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**Patentanmeldung Nr. Patent application No. Demande de brevet n°**

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Im Auftrag

For the President of the European Patent Office

Le Président de l'Office européen des brevets
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If no title is shown please refer to the description.
Si aucun titre n'est indiqué se referer à la description.)

Specified deleted sequences of tubercle bacilli and their uses in diagnostics and vaccines .

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The present invention pertains to the field of biology, more particularly the subject of the present invention is the identification of a nucleotide sequence which make it possible in particular to distinguish an infection resulting from *Mycobacterium tuberculosis* from an infection resulting from *Mycobacterium africanum, Mycobacterium canetti, Mycobacterium microti, Mycobacterium bovis, Mycobacterium bovis BCG.* The subject of the present invention is also a method for detecting the sequences in question by the products of expression of these sequences and the kits for carrying out these methods. Finally, the subject of the present invention is novel vaccines.

Despite more than a century of research since the discovery of *Mycobacterium tuberculosis,* the aetiological agent of tuberculosis, this disease remains one of the major causes of human mortality. *M. tuberculosis* is expected to kill 3 million people annually (Snider, 1989 Rev. Inf. Dis. S335) and the number of new people getting infected each year is rising and is estimated at 8.8 million. Although the majority of these are in developing countries, the disease is assuming renewed importance in the western countries due to the increasing number of homeless people, the impact of the AIDS epidemic, the changing global migration, and the travel patterns.

Early tuberculosis often goes unrecognized in an otherwise healthy individual. Classical initial methods of diagnosis include examination of a sputum smear under a microscope for acid-fast mycobacteria and an x-ray of the lungs. However, in a vast majority of cases the sputum smear examination is negative for Mycobacteria in the early stages of the disease, and lung changes may not be obvious on an x-ray until several months following infection. Another complicating factor is that acid-fast bacteria in a sputum smear may often be other species of mycobacteria. Antibiotics used for treating tuberculosis have considerable side effects, and must be taken as a combination of three or more drugs for a six to twelve month period. In addition, the possibility of inducing the appearance of drug resistant tuberculosis prevents therapy from being administered without solid evidence to support the diagnosis. Currently the only absolutely reliable method of diagnosis is based on culturing *M. tuberculosis* from the clinical specimen and identifying it morphologically and biochemically. This usually takes anywhere from three to six weeks, during which time a patient may become seriously ill and infect other individuals. Therefore, a rapid test capable of reliably detecting the presence of *M. tuberculosis* is vital for the early detection and treatment. Several molecular tests have been developed recently for the rapid detection and identification of *M. tuberculosis,* such as the Gen-Probe "Amplified *Mycobacterium tuberculosis* Direct Test"; this test amplifies *M. tuberculosis* 16S ribosomal RNA from

respiratory specimens and uses a chemiluminescent probe to detect the amplified product with a reported sensitivity of about 91%. The discovery of the IS6110 insertion element (Cave et al., Eisenach *et al.*,1990 J. Infectious Diseases 161:977-981; Thierry *et al.* 1990 J. Clin. Microbiol. 28: 2668-2673) and the belief that this element may only be present in *Mycobacterium* complex (*M. tuberculosis, M.bovis, M.bovis*-BCG, *M. africanum and M.microti*) spawned a whole series of rapid diagnostic strategies (Brisson-Noel *et al.*, 1991 Lancet 338: 364-366; Clarridge *et al.*1993, J. Clin. Microbiol. 31 :2049-2056 ; Cormican et al. 1992 J. Clin. Pathology 1992, 45 : 601-604 ; Cousins et al., 1992 J. Clin. Microbiol. 30 : 255-258 ; Del Portillo et al. 1991 J. Clin. Microbiol. 29 : 2163-2168 ; Folgueira et al., 1994 Neurology 44 :1336-1338 ; Forbes et al. 1993, J.Clin.Microbiol. 31 :1688-1694 ; Hermans et al. 1990 J. Clin. Microbiol. 28 :1204-1213 ;, Kaltwasser et al. 1993 Mol. Cell. Probes 7 : 465-470 ; Kocagoz et al. 1993 J. Clin. Microbiol. 31 :1435-1438 ; Kolk et al. 1992 J.Clin.Microbiol. 30 : 2567-2575 ; Kox et al. 1994 J.Clin.Microbiol. 32 :672-678 ; Liu et al. 1994 Neurology 44 :1161-1164 ; Miller et al. 1994 J. Clin.Microbiol. 32 : 393-397 ; Reischl et al. 1994 Biotechniques 17 :844-845 ; Schluger et al. 1994 Chest 105 :1116-1121 ; Shawar et al. 1993 J. Clin. Microbiol. 31: 61-65; Wilson et al 1993 J.Clin.Microbiol. 28: 2668-2673). These tests employ various techniques to extract DNA from the sputum. PCR is used to amplify IS6110 DNA sequences from the extracted DNA. The successful amplification of this DNA is considered to be an indicator of the presence of *M.tuberculosis* infection. U.S. Pat. Nos. 5,168,039 and 5,370,998 have been issued to Crawford *et al.* for the IS6110 based detection of tuberculosis. European patent EP 0,461,045 has been issued to Guesdon for the IS6110 based detection of tuberculosis.

Thus, these molecular assays used to detect *M. tuberculosis* depend on the IS6110 insertion sequence (about 10 copies) or the 16S ribosomal RNA (thousands of copies). However, these methods do not provide any information regarding the sub-type of the mycobacteria. Indeed several dozen species of Mycobacteria are known, and most are non-pathogenic for humans; tuberculosis is usually caused by infection due to *M. tuberculosis*, with a few cases being caused by *M. bovis*, *M.canettii*, and *M. africanum*. In order to choose an appropriate treatment and to conduct epidemiological investigations it is absolutely necessary to be able to rapidly and accurately identify isolates, i.e to distinguish the sub-type of mycobacteria of the *Mycobcaterium* complex, originating from potential tuberculosis patients. That's the problem the present invention intends to solve.

The present invention provides an isolated or purified nucleic acid wherein said nucleic acid is selected from the group consisting of:

a) SEQ ID N°1, named TbD1 (*M. tuberculosis* specific deletion 1);

b) Nucleic acid having a sequence fully complementary to SEQ ID N°1.

c) Nucleic acid fragment comprising at least 8, 15, 20, 25, 30, 50, 100, 250, 500, 750, 1000, 1500, 2000, 2500, 3000 consecutive nucleotides of SEQ ID N°1;

d) Nucleic acid having at least 90% sequence identity after optimal alignment with a sequence defined in a) or b);

e) Nucleic acid that hybridizes under stringent conditions with the nucleic acid defined in a) or b);

As used herein, the terms « isolated » and « purified » according to the invention refer to a level of purity that is achievable using current technology. The molecules of the invention do not need to be absolutely pure (i.e., contain absolutely no molecules of other cellular macromolecules), but should be sufficiently pure so that one of ordinary skill in the art would recognize that they are no longer present in the environment in which they were originally found (i.e., the cellular middle). Thus, a purified or isolated molecule according to the present invention is one that have been removed from at least one other macromolecule present in the natural environment in which it was found. More preferably, the molecules of the invention are essentially purified and/or isolated, which means that the composition in which they are present is almost completely, or even absolutely, free of other macromolecules found in the environment in which the molecules of the invention are originally found. Isolation and purification thus does not occur by addition or removal of salts, solvents, or elements of the periodic table, but must include the removal of at least some macromolecules. The nucleic acids encompassed by the invention are purified and/or isolated by any appropriate technique known to the ordinary artisan. Such techniques are widely known, commonly practiced, and well within the skill of the ordinary artisan. As used herein, the term " nucleic acid" refers to a polynucleotide sequence such as a single or double stranded DNA sequence, RNA sequence, cDNA sequence; such a polynucleotide sequence has been isolated, purified or synthesized and may be constituted with natural or non natural nucleotides. In a preferred embodiment the DNA molecule of the invention is a double stranded DNA molecule. As used herein, the terms "nucleic acid", "oligonucleotide", "polynucleotide" have the same meaning and are used indifferently.

By the term "*Mycobacterium* complex" as used herein, it is meant the complex of mycobacteria causing tuberculosis which are *Mycobacterium tuberculosis*, *Mycobacterium*

*bovis*, *Mycobacterium africanum*, *Mycobacterium microti*, *Mycobacterium canettii* and the vaccine strain *Mycobacterium bovis* BCG.

The present invention encompasses not only the entire sequence SEQ ID N°1, its complement, and its double-stranded form, but any fragment of this sequence, its complement, and its double-stranded form.

In embodiments, the fragment of SEQ ID N°1 comprises at least approximately 8 nucleotides. For example, the fragment can be between approximately 8 and 30 nucleotides and can be designed as a primer for polynucleotide synthesis. In another preferred embodiment, the fragment of SEQ ID N°1 comprises between approximately 1,500 and approximately 2,500 nucleotides, and more preferably 2153 nucleotides corresponding to SEQ ID N°4. As used herein, "nucleotides" is used in reference to the number of nucleotides on a single-stranded nucleic acid. However, the term also encompasses double-stranded molecules. Thus, a fragment comprising 2,153 nucleotides according to the invention is a single-stranded molecule comprising 2,153 nucleotides, and also a double stranded molecule comprising 2153 base pairs (bp).

In a preferred embodiment, the nucleic acid fragment of the invention is specifically deleted in the genome of *Mycobacterium tuberculosis*, excepted in *Mycobacterium tuberculosis* strain having the mutation CTG -> CGG at codon 463 of gene KatG and/or having no or very few IS6110 sequences inserted in their genome and present in the genome of *Mycobacterium africanum*, *Mycobacterium canetti*, *Mycobacterium microti*, *Mycobacterium bovis*, *Mycobacterium bovis BCG*. By the term "few IS6110 sequences inserted in the genome", it is meant less than ten copies in the genome of *M. tuberculosis*, more preferably less than 5 copies, for example less than two copies.

The nucleic acid fragment of the invention is preferably selected from the group consisting of:

a) SEQ ID N°4;

b) Nucleic acid having a sequence fully complementary to SEQ ID N°4.

c) Nucleic acid fragment comprising at least 20, 25, 30, 50, 100, 250, 500, 750, 1000, 1500, 2000, 2500, 3000 consecutive nucleotides of SEQ ID N°4;

d) Nucleic acid having at least 90% sequence identity after optimal alignment with a sequence defined in a) or b);

e) Nucleic acid that hybridizes under stringent conditions with the nucleic acid defined in a) or b); In embodiments, the stringent conditions under which a sequence according to the invention is determined are conditions which are no less stringent than 5xSSPE,

2xDenhardt's solution, and 0.5% (w/v) sodium dodecyl sulfate at 65°C. More stringent conditions can be utilized by the ordinary artisan, and the proper conditions for a given assay can be easily and rapidly determined without undue or excessive experimentation. As an illustrative embodiment, the stringent hybridization conditions used in order to specifically detect a polynucleotide according to the present invention are advantageously the following: pre-hybridization and hybridization are performed at 65°C in a mixture containing:

- 5X SSPE (1X SSPE is 3 M NaCl, 30 mM tri-sodium citrate)
- 2X Denhardt's solution
- 0.5% (w/v) sodium dodecyl sulfate (SDS)
- 100 $\mu g\ ml^{-1}$ salmon sperm DNA.

The washings are performed as follows:

- two washings at laboratory temperature (approximately 21-25°C) for 10 min. in the presence of 2X SSPE and 0.1% SDS; and
- one washing at 65°C for 15 min. in the presence of 1X SSPE and 0.1% SDS.

The invention also encompasses the isolated or purified nucleic acid of the invention wherein said nucleic acid comprises at least a deletion of a nucleic acid fragment as defined above.

Polynucleotides of the invention can be characterized by the percentage of identity they show with the sequences disclosed herein. For example, polynucleotides having at least 90% identity with the polynucleotides of the invention, particularly those sequences of the sequence listing, are encompassed by the invention. Preferably, the sequences show at least 90% identity with those of the sequence listing. More preferably, they show at least 92% identity, for example 95% or 99% identity. The skilled artisan can identify sequences according to the invention through the use of the sequence analysis software BLAST (see for example, Coffin et al., eds., "*Retroviruses*", Cold Spring Harbor Laboratory Press, pp. 723-755). Percent identity is calculated using the BLAST sequence analysis program suite, Version 2, available at the NCBI (NIH). All default parameters are used. BLAST (Basic Local Alignment Search Tool) is the heuristic search algorithm employed by the programs blastp, blastn, blastx, tblastn and tblastx, all of which are available through the BLAST analysis software suite at the NCBI. These programs ascribe significance to their findings using the statistical methods of Karlin and Altschul (1990, 1993) with a few enhancements. Using this publicly available sequence analysis program suite, the skilled artisan can easily identify polynucleotides according to the present invention.

It is well within the skill of the ordinary artisan to identify regions of the nucleic acid sequence of the invention, which would be useful as a probe, primer, or other experimental, diagnostic, or therapeutic aid. For example, the ordinary artisan could utilize any of the widely available sequence analysis programs to select regions (fragments) of these sequences that are useful for hybridization assays such as Southern blots, Northern blots, DNA binding assays, and/or *in vitro, in situ,* or *in vivo* hybridizations. Additionally, the ordinary artisan, with the sequences of the present invention, can utilize widely available sequence analysis programs to identify regions that can be used as probes and primers, as well as for design of anti-sense molecules. The only practical limitation on the fragment chosen by the ordinary artisan is the ability of the fragment to be useful for the purpose for which it is chosen. For example, if the ordinary artisan wished to choose a hybridization probe, he would know how to choose one of sufficient length, and of sufficient stability, to give meaningful results. The conditions chosen would be those typically used in hybridization assays developed for nucleic acid fragments of the approximate chosen length.

Thus, the present invention provides short oligonucleotides, such as those useful as probes and primers. In embodiments, the probe and/or primer comprises 8 to 30 consecutive nucleotides of the polynucleotide according to the invention or the polynucleotide complementary thereto. Advantageously, a fragment as defined herein has a length of at least 8 nucleotides, which is approximately the minimal length that has been determined to allow specific hybridization. Preferably the nucleic fragment has a length of at least 12 nucleotides and more preferably 20 consecutive nucleotides of any of SEQ ID NO:1 or SEQ ID NO:3. The sequence of the oligonucleotide can be any of the many possible sequences according to the invention. Preferably, the sequence is selected from the following group SEQ ID No. 13, SEQ ID No. 14, SEQ ID N°15, SEQ ID N°16, SEQ ID N°17, SEQ ID N°18. More precisely, the primers pairs SEQ ID N°13/SEQ ID N°14 and SEQ ID N°15/SEQ ID N°16 are specific for nucleic acid fragment SEQ ID N°4. The primers pair SEQ ID N°17/SEQ ID No. 18 is specific for nucleic acid sequence SEQ ID N°1 and are flanking the nucleic acid fragment of SEQ ID N°4.

Thus, the polynucleotides of SEQ ID N°1 and SEQ ID N°4, and their fragments, can be used to select nucleotide primers, notably for an amplification reaction, such as the amplification reactions further described.

PCR is described in US Patent No. 4,683,202, which is incorporated in its entirety herein. The amplified fragments may be identified by agarose or polyacrylamide gel electrophoresis, by a capillary electrophoresis, or alternatively by a chromatography

technique (gel filtration, hydrophobic chromatography, or ion exchange chromatography). The specificity of the amplification can be ensured by a molecular hybridization using as nucleic probes the polynucleotides of SEQ ID N°1 or SEQ ID N°4, and their fragments, oligonucleotides that are complementary to these polynucleotides or fragments thereof, or their amplification products themselves, or even bt DNA sequencing.

The following other techniques related to nucleic acid amplification may also be used and are generally preferred to the PCR technique. The Strand Displacement Amplification (SDA) technique is an isothermal amplification technique based on the ability of a restriction enzyme to cleave one of the strands at a recognition site (which is under a hemiphosphorothioate form) and on the property of a DNA polymerase to initiate the synthesis of a new strand from the 3'OH end generated by the restriction enzyme and on the property of this DNA polymerase to displace the previously synthesized strand being localized downstream. The SDA amplification technique is more easily performed than PCR (a single thermostatted water bath device is necessary), and is faster than the other amplification methods. Thus, the present invention also comprises using the nucleic acid fragments according to the invention (primers) in a method of DNA or RNA amplification according to the SDA technique.

When the target polynucleotide to be detected is a RNA, for example a mRNA, a reverse transcriptase enzyme will be used before the amplification reaction in order to obtain a cDNA from the RNA contained in the biological sample. The generated cDNA is subsequently used as the nucleic acid target for the primers or the probes used in an amplification process or a detection process according to the present invention.

The non-labeled polynucleotides or oligonucleotides of the invention can be directly used as probes. Nevertheless, the polynucleotides or oligonucleotides are generally labeled with a radioactive element ($^{32}P$, $^{35}S$, $^{3}H$, $^{125}I$) or by a non-isotopic molecule (for example, biotin, acetylaminofluorene, digoxigenin, 5-bromodesoxyuridine, fluorescein) in order to generate probes that are useful for numerous applications. Examples of non-radioactive labeling of nucleic acid fragments are described in French patent N° FR 78 10975 and by Urdea *et al.* (1988, *Nucleic Acids Research* 11:4937-4957) or Sanchez-Pescador *et al.* (1988, *J. Clin. Microbiol.* 26(10):1934-1938), the disclosures of which are hereby incorporated in their entirety. Other labeling techniques can also be used, such as those described in French patents FR 2 422 956 and FR 2 518 755. The hybridization step may be performed in different ways. See, for example, Matthews *et al.*, 1988, *Anal. Biochem.* 169:1-25. A general method comprises immobilizing the nucleic acid that has been extracted from the biological

sample on a substrate (for example, nitrocellulose, nylon, polystyrene) and then incubating, in defined conditions, the target nucleic acid with the probe. Subsequent to the hybridization step, the excess amount of the specific probe is discarded and the hybrid molecules formed are detected by an appropriate method (radioactivity, fluorescence or enzyme activity measurement, etc.).

Amplified nucleotide fragments are useful, among other things, as probes used in hybridization reactions in order to detect the presence of one polynucleotide according to the present invention or in order to detect mutations. The primers may also be used as oligonucleotide probes to specifically detect a polynucleotide according to the invention.

The oligonucleotide probes according to the present invention may also be used in a detection device comprising a matrix library of probes immobilized on a substrate, the sequence of each probe of a given length being localized in a shift of one or several bases, one from the other, each probe of the matrix library thus being complementary to a distinct sequence of the target nucleic acid. Optionally, the substrate of the matrix may be a material able to act as an electron donor, the detection of the matrix positions in which an hybridization has occurred being subsequently determined by an electronic device. Such matrix libraries of probes and methods of specific detection of a target nucleic acid is described in the European patent application N° EP-0 713 016 (Affymax technologies) and also in the US patent N° US-5,202,231 (Drmanac). Since almost the whole length of a mycobacterial chromosome is covered by BAC-based genomic DNA library (i.e. 97% of the *M. tuberculosis* chromosome is covered by the BAC library I-1945), these DNA libraries will play an important role in a plurality of post-genomic applications, such as in mycobacterial gene expression studies where the canonical set of BACs could be used as a matrix for hybridization studies. Thus it is also in the scope of the invention to provide a nucleic acid chips, more precisely a DNA chips or a protein chips that respectively comprises a nucleic acid or a polypeptide of the invention.

The present invention is also providing a vector comprising the isolated DNA molecule of the invention. A " vector " is a replicon in which another polynucleotide segment is attached, so as to bring the replication and/or expression to the attached segment. A vector can have one or more restriction endonuclease recognition sites at which the DNA sequences can be cut in a determinable fashion without loss of an essential biological function of the vector, and into which a DNA fragment can be spliced in order to bring about its replication and cloning. Vectors can further provide primer sites (e.g. for PCR), transcriptional and/or translational initiation and/or regulation sites, recombinational signals,

replicons, selectable markers, etc. Beside the use of homologous recombination or restriction enzymes to insert a desired DNA fragment into the vector, UDG cloning of PCR fragments (US Pat. No. 5,334,575), T:A cloning, and the like can also be applied. The cloning vector can further contain a selectable marker suitable for use in the identification of cells transformed with the cloning vector.

The vector can be any useful vector known to the ordinary artisan, including, but not limited to, a cloning vector, an insertion vector, or an expression vector. Examples of vectors include plasmids, phages, cosmids, phagemid, yeast artificial chromosome (YAC), bacterial artificial chromosome (BAC), human artificial chromosome (HAC), viral vector, such as adenoviral vector, retroviral vector, and other DNA sequences which are able to replicate or to be replicated *in vitro* or in a host cell, or to convey a desired DNA segment to a desired location within a host cell. According to a preferred embodiment of the invention, the recombinant vector is a BAC pBeloBAC11 in which the genomic region of *Mycobacterium bovis-BCG* 1173P3 that spans the region corresponding to the locus 1,760,753 bp to 1,830,364 bp in the genome of *M. tuberculosis* H37Rv has been inserted into the HindIII restriction site; this recombinant vector is named X229. In this region, the inventors have demonstrated the deletion of a 2153 bp fragment in the vast majority of *M. tuberculosis* strain. That's the reason why the inventors named this region TbD1 ("*M. tuberculosis* specific deletion 1"). This 2153 bp region is flanked by the sequence GGC CTG GTC AAA CGC GGC TGG ATG CTG and AGA TCC GTC TTT GAC ACG ATC GAC G. External primers hybridizing with such sequences or the complentary sequences thereof can be used for the amplification of TbD1 region to check for the presence or the absence of the deletion of the TbD1 region. The inventors design for example the following primers:

5'- CTA CCT CAT CTT CCG GTC CA-3' (SEQ ID N° 17)

5'- CAT AGA TCC CGG ACA TGG TG-3'(SEQ ID N° 18)

In order to get a specific 500 pb probe for hybridization experiments, a PCR amplification of an internal fragment may be realized by using the plasmid X229 as a matrix. The amplification of a fragment of approximatively 500 bp internal to the TbD1 region can be performed by using the following primers:

5'- CGT TCA ACC CCA AAC AGG TA-3' (SEQ ID N° 13)

5'- AAT CGA ACT CGT GGA ACA CC-3' (SEQ ID N° 14)

The amplification of a fragment of approximatively 2,000 bp internal to the TbD1 region can be performed by using the following primers:

5'- ATT CAG CGT CTA TCG GTT GC-3' (SEQ ID N° 15)

5'- AGC AGC TCG GGA TAT CGT AG-3' (SEQ ID N° 16)

The PCR conditions are the following: denaturation 95°C 1 min, then 35 cycles of amplification [95°C during 30 seconds, 58°C during 1 min] , then elongation 72°C during 4 min.

Thus, this invention also concerns a recombinant cell host which contains a polynucleotide or recombinant vector according to the invention. The cell host can be transformed or transfected with a polynucleotide or recombinant vector to provide transient, stable, or controlled expression of the desired polynucleotide. For example, the polynucleotide of interest can be subcloned into an expression plasmid at a cloning site downstream from a promoter in the plasmid and the plasmid can be introduced into a host cell where expression can occur. The recombinant host cell can be any suitable host known to the skilled artisan, such as a eukaryotic cell or a microorganism. For example, the host can be a cell selected from the group consisting of *Escherichia coli, Bacillus subtilis,* insect cells, and yeasts. According to a preferred embodiment of the invention, the recombinant cell host is a commercially available *Escherichia coli* DH10B (Gibco) containing the BAC named X229 previously described. This *Escherichia coli* DH10B (Gibco) containing the BAC named X229 has been deposited with the Collection Nationale de Cultures de Microorganismes (CNCM), Institut Pasteur, Paris, France, on February 18th, 2002 under number CNCM I-2799.

Another aspect of the invention is the isolated or purified polypeptides encoded by a polynucleotide of the invention. The purified polypeptide comprises an amino acid sequence that is encoded by SEQ ID N° 6, N° 8, N° 10, N°12, and their fragments thereof. For example, the purified polypeptide of the invention can comprise the amino acid sequence of SEQ ID N°6, which is the amino acid sequence of the mmpL6 protein or the amino acid sequence of SEQ ID N°8 a truncated form of mmpL6. The purified polypeptide of the invention can comprise the amino acid sequence of SEQ ID NO:10, which is the amino acid sequence of the mmpS6 protein or the amino acid sequence of SEQ ID N°12 a truncated form of mmpS6.

It is now easy to produce proteins in large amounts by genetic engineering techniques through the use of expression vectors, such as plasmids, phages, and phagemids. The polypeptide of the present invention can be produced by insertion of the appropriate polynucleotide into an appropriate expression vector at the appropriate position within the vector. Such manipulation of polynucleotides is well known and widely practiced by the ordinary artisan. The polypeptide can be produced from these recombinant vectors either *in*

*vitro* or *in vivo*. All the isolated or purified nucleic acid encoding by the polypeptide of the invention are in the scope of the invention. The polypeptide of the invention is a polypeptide encoded by a polynucleotide which hybridizes to any of SEQ ID N°1 or N°4 under stringent conditions, as defined herein.

More preferably, said isolated or purified nucleic acid according the invention is selected among:

- SEQ ID N° 5 encoding the polypeptide of SEQ ID N°6;
- SEQ ID N° 7 encoding the polypeptide of SEQ ID N°8;
- SEQ ID N° 9 encoding the polypeptide of SEQ ID N°10;
- SEQ ID N° 11 encoding the polypeptide of SEQ ID N°12.

The present invention also provides a method for the discriminatory detection and identification of:

- *Mycobacterium tuberculosis* excepted *Mycobacterium tuberculosis* strain having the mutation CTG -> CGG at codon 463 of gene KatG and/or excepted *Mycobacterium tuberculosis* strain having no or very few IS6110 sequences inserted in their genome; versus,
- *Mycobacterium africanum, Mycobacterium canettii, Mycobacterium microti, Mycobacterium bovis, Mycobacterium bovis BCG* in a biological sample,

comprising the following steps:

a) isolation of the DNA from the biological sample to be analyzed or production of a cDNA from the RNA of the biological sample,

b) detection of the nucleic acid sequences of the mycobacterium present in said biological sample,

c) analysis for the presence or the absence of a nucleic acid fragment as previously described.

By a biological sample according to the present invention, it is notably intended a biological fluid, such as sputum, saliva, plasma, blood, urine or sperm, or a tissue, such as a biopsy.

Analysis of the desired sequences may, for example, be carried out by agarose gel electrophoresis. If the presence of a DNA fragment migrating to the expected site is observed, it can be concluded that the analyzed sample contained mycobacterial DNA. This analysis can also be carried out by the molecular hybridization technique using a nucleic probe. This probe will be advantageously labeled with a nonradioactive (cold probe) or radioactive element. Advantageously, the detection of the mycobacterial DNA sequences will be carried out using nucleotide sequences complementary to said DNA sequences. By way of example, they may include labeled or nonlabeled nucleotide probes; they may also

include primers for amplification. The amplification technique used may be PCR but also other alternative techniques such as the SDA (Strand Displacement Amplification) technique, the TAS technique (Transcription-based Amplification System), the NASBA (Nucleic Acid Sequence Based Amplification) technique or the TMA (Transcription Mediated Amplification) technique.

The primers in accordance with the invention have a nucleotide sequence chosen from the group comprising SEQ ID No. 13, SEQ ID No. 1, SEQ ID N°15, SEQ ID N°16, SEQ ID N°17, SEQ ID N°18. The pairs SEQ ID N°13/SEQ ID N°14 and SEQ ID N°15/SEQ ID N°16 specific for nucleic acid fragment SEQ ID N°4, and the pair SEQ ID N°17/SEQ ID N°18 specific for nucleic acid of the invention.

In a variant, the subject of the invention is also a method for the discriminatory detection and identification of:

- *Mycobacterium tuberculosis* excepted *Mycobacterium tuberculosis* strain having the mutation CTG -> CGG at codon 463 of gene katG and/or excepted *Mycobacterium tuberculosis* strain having no or very few IS6110 sequences inserted in their genome; versus,
- *Mycobacterium africanum, Mycobacterium canettii, Mycobacterium microti, Mycobacterium bovis, Mycobacterium bovis BCG* in a biological sample,

comprising the following steps:

a) bringing the biological sample to be analyzed into contact with at least one pair of primers as defined in claim 11 or 12, the DNA contained in the sample having been, where appropriate, made accessible to the hybridization beforehand,

b) amplification of the DNA of the mycobacterium,

c) visualization of the amplification of the DNA fragments.

The amplified fragments may be identified by agarose or polyacrylamide gel electrophoresis by capillary electrophoresis or by a chromatographic technique (gel filtration, hydrophobic chromatography or ion-exchange chromatography). The specification of the amplification may be controlled by molecular hybridization using probes, plasmids containing these sequences or their product of amplification. The amplified nucleotide fragments may be used as reagent in hybridization reactions in order to detect the presence, in a biological sample, of a target nucleic acid having sequences complementary to those of said amplified nucleotide fragments. These probes and amplicons may be labeled or otherwise with radioactive elements or with nonradioactive molecules such as enzymes or fluorescent elements.

The subject of the present invention is also a kit for the discriminatory detection and identification of:

- *Mycobacterium tuberculosis* excepted *Mycobacterium tuberculosis* strain having the mutation CTG -> CGG at codon 463 of gene katG and/or excepted *Mycobacterium tuberculosis* strain having no or very few IS6110 sequences inserted in their genome; versus,
- *Mycobacterium africanum, Mycobacterium canettii, Mycobacterium microti, Mycobacterium bovis, Mycobacterium bovis BCG* in a biological sample,

in a biological sample comprising the following elements:

a) at least one pair of primers as defined previously,

b) the reagents necessary to carry out a DNA amplification reaction,

c) optionally, the necessary components which make it possible to verify or compare the sequence and/or the size of the amplified fragment.

Indeed, in the context of the present invention, depending on the pair of primers used, it is possible to obtain very different results. Thus, the use of primers which are internal to the deletion, such as for example SEQ ID N°13, SEQ ID N°14, SEQ ID N°15, SEQ ID N°16, is such that no amplification product is detectable in *M. tuberculosis* and that amplification product is detectable in *Mycobacterium africanum, Mycobacterium canettii, Mycobacterium microti, Mycobacterium bovis, Mycobacterium bovis BCG, Mycobacterium tuberculosis* having the mutation CTG -> CGG at codon 463 of gene katG and/or having no or very few IS6110 sequences inserted in their genome. However, the use of primers external to the region of deletion does not necessarily give the same result, as regards for example the size of the amplified fragment, depending on the size of the deleted region in *M. tuberculosis*. Thus, the use of the pair of primers external to the deletion such as SEQ ID N° 17 and SEQ ID N° 18 is likely to give rise to an amplicon in *Mycobacterium africanum, Mycobacterium canettii, Mycobacterium microti, Mycobacterium bovis, Mycobacterium bovis BCG, Mycobacterium tuberculosis* having the mutation CTG -> CGG at codon 463 of gene katG and/or having no or very few IS6110 sequences inserted in their genome, of about 2100 bp whereas the use of the pair of primers external to the deletion will give rise in *M. tuberculosis* to an amplicon of about few bp.

More generally, the invention pertains to the use of at least one pair of primers as defined previously for the amplification of a DNA sequence from *Mycobacterium tuberculosis* or *Mycobacterium africanum, Mycobacterium canettii, Mycobacterium microti, Mycobacterium bovis, Mycobacterium bovis BCG, Mycobacterium Tuberculosis* having the

mutation CTG -> CGG at codon 463 of gene katG and/or having no or very few IS6110 sequences inserted in their genome.

The subject of the invention is also the product of expression of all or part of the nucleic acid fragment as defined previously and deleted from the genome of *Mycobacterium tuberculosis* and present in *Mycobacterium africanum, Mycobacterium canettii, Mycobacterium microti, Mycobacterium bovis, Mycobacterium bovis BCG, Mycobacterium Tuberculosis* having the mutation CTG -> CGG at codon 463 of gene katG and/or having no or very few IS6110 sequences inserted in their genome; or conversely. The expression "product of expression" is understood to mean any protein, polypeptide or polypeptide fragment resulting from the expression of all or part of the above-mentioned nucleotide sequences. Among those product of expression, one can cite the membrane proteins mmpL6, mmpS6, and their truncated or rearranged form due to the deletion of the fragment of the invention.

Indeed, the subject of the present invention is also a method for the discriminatory detection *in vitro* of antibodies directed against *Mycobacterium tuberculosis* or *Mycobacterium africanum, Mycobacterium canettii, Mycobacterium microti, Mycobacterium bovis, Mycobacterium bovis BCG, Mycobacterium tuberculosis* having the mutation CTG -> CGG at codon 463 of gene KatG and/or having no or very few IS6110 sequences inserted in their genome, in a biological sample, comprising the following steps:

a) bringing the biological sample into contact with at least one product as previously defined ,

b) detecting the antigen-antibody complex formed.

The subject of the present invention is also a method for the discriminatory detection of a vaccination with *Mycobacterium bovis* BCG or an infection by *Mycobacterium tuberculosis,* excepted *Mycobacterium tuberculosis* strain having the mutation CTG -> CGG at codon 463 of gene katG and/or excepted *Mycobacterium tuberculosis* strain having no or very few IS6110 sequences inserted in their genome in a mammal, comprising the following steps:

a) preparation of a biological sample containing cells, more particularly cells of the immune system of said mammal and more particularly T cells,

b) incubation of the biological sample of step a) with at least one product as previously defined ,

c) detection of a cellular reaction indicating prior sensitization of the mammal to said product, in particular cell proliferation and/or synthesis of proteins such as gamma-interferon. Cell proliferation may be measured, for example, by incorporating $^{3}$H-Thymidine.

The invention also relates to a kit for the *in vitro* diagnosis of an *Mycobacterium tuberculosis* infection, excepted infection with *Mycobacterium tuberculosis* strain having the mutation CTG -> CGG at codon 463 of gene katG and/or having no or very few IS6110 sequences inserted in their genome, in a mammal optionally vaccinated beforehand with *M. bovis* BCG comprising:

a) a product as previously defined ,

b) where appropriate, the reagents for the constitution of the medium suitable for the immunological reaction,

c) the reagents allowing the detection of the antigen-antibody complexes produced by the immunological reaction,

d) where appropriate, a reference biological sample (negative control) free of antibodies recognized by said product,

e) where appropriate, a reference biological sample (positive control) containing a predetermined quantity of antibodies recognized by said product.

The reagents allowing the detection of the antigen-antibody complexes may carry a marker or may be capable of being recognized in turn by a labeled reagent, more particularly in the case where the antibody used is not labeled.

The subject of the invention is also mono- or polyclonal antibodies, their chimeric fragments or antibodies, capable of specifically recognizing a product of expression in accordance with the present invention.

The present invention therefore also relates to a method for the discriminatory detection of the presence of an antigen of *Mycobacterium tuberculosis* or *Mycobacterium africanum, Mycobacterium canettii, Mycobacterium microti, Mycobacterium bovis, Mycobacterium bovis*-BCG, *Mycobacterium Tuberculosis* having the mutation CTG -> CGG at codon 463 of gene katG and/or having no or very few IS6110 sequences inserted in their genome in a biological sample comprising the following steps:

a) bringing the biological sample into contact with an antibody of the invention,

b) detecting the antigen-antibody complex formed.

The invention also relates to the kit for the discriminatory detection of the presence of an antigen of *Mycobacterium tuberculosis* or *Mycobacterium africanum, Mycobacterium canettii, Mycobacterium microti, Mycobacterium bovis, Mycobacterium bovis BCG,*

*Mycobacterium tuberculosis* having the mutation CTG -> CGG at codon 463 of gene katG and/or having no or very few IS6110 sequences inserted in their genome, in a biological sample comprising the following steps:

a) an antibody as previously claimed ,

b) the reagents for constituting the medium suitable for the immunological reaction,

c) the reagents allowing the detection of the antigen-antibody complexes produced by the immunological reaction.

The above-mentioned reagents are well known to a person skilled in the art who will have no difficulty adapting them to the context of the present invention.

The subject of the invention is also an immunological composition, characterized in that it comprises at least one product of expression in accordance with the invention.

Advantageously, the immunological composition in accordance with the invention enters into the composition of a vaccine when it is provided in combination with a pharmaceutically acceptable vehicle and optionally with one or more immunity adjuvant(s) such as alum or a representative of the family of muramylpeptides or incomplete Freund's adjuvant.

The invention also relates to a vaccine comprising at least one product of expression in accordance with the invention in combination with a pharmaceutically compatible vehicle and, where appropriate, one or more appropriate immunity adjuvant(s).

The invention also provide an in vitro method for the detection and identification of *Mycobacterium tuberculosis* excepted *Mycobacterium tuberculosis* strain having the mutation CTG -> CGG at codon 463 of gene katG and/or excepted *Mycobacterium tuberculosis* strain having no or very few IS6110 sequences inserted in their genome in a biological sample,

comprising the following steps:

a) isolation of the DNA from the biological sample to be analyzed or production of a cDNA from the RNA of the biological sample,

b) detection of the nucleic acid sequences of the mycobacterium present in said biological sample,

c) analysis for the presence or the absence of a nucleic acid fragment of the invention.

In another embodiment, the invention provides an *in vitro* method for the detection and identification of *Mycobacterium tuberculosis* excepted *Mycobacterium tuberculosis* strain having the mutation CTG -> CGG at codon 463 of gene katG and/or excepted

*Mycobacterium tuberculosis* strain having no or very few IS6110 sequences inserted in their genome in a biological sample, comprising the following steps:

a) bringing the biological sample to be analyzed into contact with at least one pair of primers selected among nucleic acid fragments of the invention, and more preferably selected among the primers chosen from the group comprising SEQ ID N°13, SEQ ID N°14, SEQ ID N°15, SEQ ID N°16, SEQ ID N°17, SEQ ID N°18, the DNA contained in the sample having been, where appropriate, made accessible to the hybridization beforehand,

b) amplification of the DNA of the mycobacterium,

c) visualization of the amplification of the DNA fragments.

The invention also provides a kit for the detection and identification of *Mycobacterium tuberculosis* excepted *Mycobacterium tuberculosis* strain having the mutation CTG -> CGG at codon 463 of gene katG and/or excepted *Mycobacterium tuberculosis* strain having no or very few IS6110 sequences inserted in their genome in a biological sample, comprising the following elements:

a) at least one pair of primers selected among nucleic acid fragments of the invention, and more preferably selected among the primers chosen from the group comprising SEQ ID N°13, SEQ ID N°14, SEQ ID N°15, SEQ ID N°16, SEQ ID N°17, SEQ ID N°18,

b) the reagents necessary to carry out a DNA amplification reaction,

c) optionally, the necessary components which make it possible to verify or compare the sequence and/or the size of the amplified fragment.

The invention also relates to a method for the detection *in vitro* of antibodies directed against *Mycobacterium tuberculosis* excepted *Mycobacterium Tuberculosis* having the mutation CTG -> CGG at codon 463 of gene KatG and/or having no or very few IS6110 sequences inserted in their genome, in a biological sample, comprising the following steps:

a) bringing the biological sample into contact with at least one product as defined previously,

b) detecting the antigen-antibody complex formed.

It is also a goal of the invention to use the TbD1 deletion as a as a genetic marker for the differentiation of *Mycobacterium* strain of *Mycobacterium tuberculosis* complex.

It is also a goal of the invention to use mmpL6$^{551}$ polymorphism as a genetic marker (see SEQ ID N°20) for the differentiation of *Mycobacterium* strain of *Mycobacterium tuberculosis* complex.

The use of such genetic marker(s) in association with at least one genetic markers selected among RD1, RD2, RD3, RD4, RD5, RD6, RD7, RD8, RD9, RD10, Rd11, RD13, RD14, RvD1, RvD2, RvD3, RvD4, RvD5, $katG^{463}$, $gyrA^{95}$, $oxyR'^{285}$, $pncA^{57}$ allows the differentiation of Mycobacterium strain of Mycobacterium Tuberculosis complex (see example 4).

The present invention provides an *in vitro* method for the detection and identification of *Mycobacteria* from the *Mycobacterium Tuberculosis* complex in a biological sample, comprising the following steps:

a) analysis for the presence or the absence of a nucleic acid fragment of the sequence TbD1, and

b) analysis of at least one additional genetic marker selected among RD1, RD2, RD3, RD4, RD5, RD6, RD7, RD8, RD9, RD10, Rd11, RD13, RD14, RvD1, RvD2, RvD3, RvD4, RvD5, $katG^{463}$, $gyrA^{95}$, $oxyR'^{285}$, $pncA^{57}$.

In a preferred embodiment, two additional markers are used, preferably RD4 and RD9. The analysis is performed by a technique selected among sequence hybridization, nucleic acid amplification, antigen-antibody complex.

It is also a goal of the present invention to provide a kit for the detection and identification of *Mycobacteria* from the *Mycobacterium Tuberculosis* complex in a biological sample comprising the following elements:

a) at least one pair of primers selected among nucleic acid fragments of the invention, and more preferably selected among the primers chosen from the group comprising SEQ ID N°13, SEQ ID N°14, SEQ ID N°15, SEQ ID N°16, SEQ ID N°17, SEQ ID N°18,

b) at least one pair of primers specific of the genetic markers selected among RD1, RD2, RD3, RD4, RD5, RD6, RD7, RD8, RD9, RD10, Rd11, RD13, RD14, RvD1, RvD2, RvD3, RvD4, RvD5, $katG^{463}$, $gyrA^{95}$, $oxyR'^{285}$, $pncA^{57}$.

**c)** the reagents necessary to carry out a DNA amplification reaction,

**d)** optionally, the necessary components which make it possible to verify or compare the sequence and/or the size of the amplified fragment.

In a preferred embodiment, the kit comprises the following elements:

a) at least one pair of primers selected among nucleic acid fragments of the invention, and more preferably selected among the primers chosen from the group comprising SEQ ID N°13, SEQ ID N°14, SEQ ID N°15, SEQ ID N°16, SEQ ID N°17, SEQ ID N°18,

b) one pair of primers specific of the genetic markers RD4,

c) one pair of primers specific of the genetic markers RD9,

d) the reagents necessary to carry out a DNA amplification reaction,

e) optionally, the necessary components which make it possible to verify or compare the sequence and/or the size of the amplified fragment.

The figures and examples presented below are provided as further guide to the practitioner of ordinary skill in the art and are not to be construed as limiting the invention in anyway.

## FIGURES

**Figure 1** : Amplicons obtained from strains that have the indicated genomic region present or deleted. Sizes of amplicons in each group are uniform. Numbers correspond to strain designation used in Kremer et al. (1999, J. Clin Microbiol. 37: 2607-2618) (Ref. 8) and Supply et al (2001, J. Clin. Microbiol. 39: 3563-3571) (ref.9).

**Figure. 2**: Sequences in the TbD1 region obtained from strains of various geographic regions.
* refers to groups based on *katG*$^{c463}$/*gyrA*$^{c95}$ sequence polymorphism defined by Sreevatsan and colleagues (Ref. 2). Numbers correspond to strain designation used in Kremer et al. (1999, J. Clin Microbiol. 37: 2607-2618) (Ref. 8) and Supply et al (2001, J. Clin. Microbiol. 39: 3563-3571) (ref.9).

**Figure 3**: Spoligotypes of selected *M. tuberculosis* and *M. bovis* strains. Numbers correspond to strain designation used in Kremer et al. (1999, J. Clin Microbiol. 37: 2607-2618) (Ref. 8) and Supply et al (2001, J. Clin. Microbiol. 39: 3563-3571) (ref.9).

**Figure 4**: Scheme of the proposed evolutionary pathway of the tubercle bacilli illustrating successive loss of DNA in certain lineages (grey boxes) The scheme is based on presence or absence of conserved deleted regions and on sequence polymorphisms in five selected genes.

Note that the distances between certain branches may not correspond to actual phylogenetic differences calculated by other methods.

Blue arrows indicate that strains are characterized by *katG*$^{c463}$ CTG (Leu), *gyrA*$^{c95}$ ACC (Thr), typical for group 1 organisms. Green arrows indicate that strains belong to group 2 characterized by *katG*$^{c463}$ CGG (Arg), *gyrA*$^{c95}$ ACC (Thr). The red arrow indicates that strains belong to group 3, charcterized by *katG*$^{c463}$ CGG (Arg), *gyrA*$^{c95}$ AGC (Ser), as defined by Sreevatsan and colleagues (Sreevastan et al., 1997 Proc. Natl. Acad.Sci USA 151: 9869-9874) (Ref. 2).

# EXAMPLES

## 1. MATERIAL AND METHODS:

**1.1. Bacterial Strains:** The 100 *M. tuberculosis* complex strains comprised 46 *M. tuberculosis* strains isolated in 30 countries, 14 *M. africanum* strains, 28 *M. bovis* strains originating in 5 countries, 2 *M. bovis* BCG vaccine strains (Pasteur and Japan), 5 *M. microti* strains, and 5 *M. canettii* strains. The strains were isolated from human and animal sources and were selected to represent a wide diversity including 60 strains that have been used in a multi-center study (8). The *M. africanum* strains were retrieved from the collection of the Wadsworth Center, New York State Department of Health, Albany, New York, whereas the majority of the *M. bovis* isolates came from the collection of the University of Zaragoza, Spain. Four *M. canettii* strains are from the culture collection of the Institut Pasteur, Paris, France. The strains have been extensively characterized by reference typing methods, i.e. IS*6110* restriction fragment length polymorphism (RFLP) typing and spoligotyping. *M. tuberculosis* H37Rv, *M. tuberculosis* H37Ra, *M. tuberculosis* CDC1551, *M. bovis* AF2122/97, *M. microti* OV254, and *M. canettii* CIPT 140010059 were included as reference strains. DNA was prepared as previously described (10).

**1.2. Genome comparisons and primer design**

For preliminary genome comparisons between *M. tuberculosis* and *M. bovis* websites http://genolist.pasteur.fr/TubercuList/ and http://www.sanger.ac.uk/Projects/M_bovis/ as well as inhouse databases were used. For primer design, sequences inside or flanking RD and RvD regions were obtained from the same websites. Primers were designed using the

primer 3 website http://www-genome.wi.mit.edu/cgi-bin/primer/primer3_www.cgi that would amplify ca. 500 base pair fragments in the reference strains (Table 1).

**1.3. RD-PCR analysis**

Reactions were performed in 96 well plates and contained per reaction 1.25 µl of 10 x PCR buffer (600mM Tris HCl pH 8.8, 20 mM $MgCl_2$, 170 mM $(NH4)_2SO_4$, 100 mM β-mercaptoethanol), 1.25 µl 20mM nucleotide mix, 50 nM of each primer, 1-10 ng of template DNA, 10% DMSO, 0.2 units *Taq* polymerase (Gibco-BRL) and sterile distilled water to 12.5 µl. Thermal cycling was performed on a PTC-100 amplifier (MJ Inc.) with an initial denaturation step of 90 seconds at 95°C, followed by 35 cycles of 30 seconds at 95°C, 1 min at 58°C, and 4 min at 72°C.

**1.4. Sequencing of junction regions (RDs, TbD1,) *katG, gyrA, oxyR* and *pncA* genes**

PCR products were obtained as described above, using primers listed in Table 1.

For primer elimination, 6 µl PCR product was incubated with 1 unit of Shrimp Alkaline phosphatase (USB), 10 units of exonuclease I (USB), and 2 µl of 5 x buffer (200mM Tris HCl pH 8.8, 5mM $MgCl_2$) for 15 min at 37°C and then for 15 min at 80°C. To this reaction mixture 2 µl of Big Dye sequencing mix (Applied Biosystems), 2 µl (2µM) of primer and 3 µl of 5 x buffer (5mM $MgCl_2$, 200mM Tris HCl pH 8.8) were added and 35 cycles (96°C for 30 sec; 56°C for 15 sec; 60°C for 4 min) performed in a thermocycler (MJ-research Inc., Watertown, MA). DNA was precipitated using 80 µl of 76% ethanol, centrifuged, rinsed with 70% ethanol, and dried. Reactions were dissolved in 2 µl of formamide/EDTA buffer, denatured and loaded onto 48 cm, 4 % polyacrylamide gels and electrophoresis performed on 377 automated DNA sequencers (Applied Biosystems) for 10 to 12 h. Alternatively, reactions were dissolved in 0.3 mM EDTA buffer and subjected to automated sequencing on a 3700 DNA sequencer (Applied Biosystems). Reactions generally gave between 500-700 bp of unambiguous sequence.

**1.5. Accession Numbers**

The sequence of the TbD1 region from the ancestral *M. tuberculosis* strain No. 74 (Ref. 8) containing genes *mmpS6* and *mmpL6* was deposited in the EMBL database under accession No. AJ426486. Sequences bordering RD4, RD7, RD8, RD9 and RD10 in BCG are available under accession numbers AJ003103, AJ007301, AJ131210, Y18604, and AJ132559, respectively.

## 2. EXPERIMENTAL DATA:

The distribution of 20 variable regions resulting from insertion-deletion events in the genomes of the tubercle bacilli has been evaluated in a total of 100 strains of *Mycobacterium tuberculosis, M. africanum, M. canettii, M. microti* and *M. bovis*. This approach showed that the majority of these polymorphisms did not occur independently in the different strains of the *M. tuberculosis* complex but, rather, result from ancient, irreversible genetic events in common progenitor strains. Based on the presence or absence of an *M. tuberculosis* specific deletion (TbD1), *M. tuberculosis* strains can be divided into ancestral and "modern" strains, the latter comprising representatives of major epidemics like the Beijing, Haarlem and African *M. tuberculosis* clusters. Furthermore, successive loss of DNA, reflected by RD9 and other subsequent deletions, was identified for an evolutionary lineage represented by *M. africanum, M. microti* and *M. bovis* that diverged from the progenitor of the present *M. tuberculosis* strains before TbD1 occurred. These findings contradict the often-presented hypothesis that *M. tuberculosis*, the etiological agent of human tuberculosis evolved from *M. bovis*, the agent of bovine disease. *M. canettii* and ancestral *M. tuberculosis* strains lack none of these deleted regions and therefore appear to be direct descendants of tubercle bacilli that existed before the *M. africanum*→ *M. bovis* lineage separated from the *M. tuberculosis* lineage. This suggests that the common ancestor of the tubercle bacilli resembled *M. tuberculosis* or *M. canettii* and could well have been a human pathogen already.

The mycobacteria grouped in the *M. tuberculosis* complex are characterized by 99.9% similarity at the nucleotide level and identical 16S rRNA sequences (1, 2) but differ widely in terms of their host tropisms, phenotypes and pathogenicity. Assuming that they are all derived from a common ancestor, it is intriguing that some are exclusive human (*M. tuberculosis, M. africanum, M. canettii*) or rodent pathogens (*M. microti*) whereas others have a wide host spectrum (*M. bovis*). What was the genetic organization of the last common ancestor of the tubercle bacilli and in which host did it live? Which genetic events may have contributed to the fact that the host spectrum is so different and often specific? Where and when did *M. tuberculosis* evolve? Answers to these questions are important for a better understanding of the pathogenicity and the global epidemiology of tuberculosis and may help to anticipate future trends in the spread of the disease.

Because of the unusually high degree of conservation in their housekeeping genes it has been suggested that the members of the *M. tuberculosis* complex underwent an evolutionary bottleneck at the time of speciation, estimated to have occurred roughly 15,000

– 20,000 years ago (2). It also has been speculated that *M. tuberculosis*, the most widespread etiological agent of human tuberculosis has evolved from *M. bovis*, the agent of bovine tuberculosis, by specific adaptation of an animal pathogen to the human host (3). However, both hypotheses were proposed before the whole genome sequence of *M. tuberculosis* (4) was available and before comparative genomics uncovered several variable genomic regions in the members of the *M. tuberculosis* complex. Differential hybridization arrays identified 14 regions (RD1 –14) ranging in size from 2 to 12.7 kb that were absent from BCG Pasteur relative to *M. tuberculosis* H37Rv (5, 6). In parallel, six regions, RvD1-5, and TbD1, that were absent from the *M. tuberculosis* H37Rv genome relative to other members of the *M. tuberculosis* complex were revealed by comparative genomics approaches employing pulsed-field gel electrophoresis (PFGE) techniques (5, 7) and *in silico* comparisons of the near complete *M. bovis* AF2122/97 genome sequence and the *M. tuberculosis* H37Rv sequence.

In the present study the inventors have analyzed the distribution of these 20 variable regions situated around the genome (Table 1) in a representative and diverse set of 100 strains belonging to the *M. tuberculosis* complex. The strains were isolated from different hosts, from a broad range of geographic origins, and exhibit a wide spectrum of typing characteristics like IS*6110* and spoligotype hybridization patterns or variable-number tandem repeats of mycobacterial interspersed repetitive units (MIRU-VNTR) (8, 9). The inventors have found striking evidence that deletion of certain variable genomic regions did not occur independently in the different strains of the *M. tuberculosis* complex and, assuming that there is little or no recombination of chromosomal segments between the various lineages of the complex, this allows the inventors to propose a completely new scenario for the evolution of the *M. tuberculosis* complex and the origin of human tuberculosis.

**Variable genomic regions and their occurrence in the members of the *M. tuberculosis* complex.**

The PCR screening assay for the 20 variable regions (Table 1) within 46 *M. tuberculosis*, 14 *M. africanum*, 5 *M. canettii*, 5 *M. microti*, 28 *M. bovis* and 2 BCG strains employed oligonucleotides internal to known RDs and RvDs, as well as oligonucleotides flanking these regions (Table 1). This approach generated a large data set that was robust, highly reliable, and internally controlled since PCR amplicons obtained with the internal

primer pair correlated with the absence of an appropriately sized amplicon with the flanking primer-pair, and *vice-versa*.

According to the conservation of junction sequences flanking the variable regions three types of regions were distinguished, each having different importance as an evolutionary marker. The first type included mobile genetic elements, like the prophages phiRv1 (RD3) and phiRv2 (RD11) and insertion sequences IS*1532* (RD6) and IS*6110* (RD5), whose distribution in the tubercle bacilli was highly divergent (Table 2). The second type of deletion is mediated by homologous recombination between adjacent IS*6110* insertion elements resulting in the loss of the intervening DNA segment (RvD2, RvD3, RvD4, and RvD5 (7)) and is variable from strain to strain (Table 2).

The third type includes deletions whose bordering genomic regions typically do not contain repetitive sequences. Often this type of deletion occurred in coding regions resulting in the truncation of genes that are still intact in other strains of the *M. tuberculosis* complex. The exact mechanism leading to this type of deletion remains obscure, but possibly rare strand slippage errors of DNA polymerase may have contributed to this event. As shown in detail below, RD1, RD2, RD4, RD7, RD8, RD9, RD10, RD12, RD13, RD14, and TbD1 are representatives of this third group whose distribution among the 100 strains allows us to propose an evolutionary scenario for the members of the *M. tuberculosis* complex, that identified *M. tuberculosis* and/or *M. canettii* as most closely related to the common ancestor of the tubercle bacilli.

**2.1. *M. tuberculosis* strains:**

Investigation of the 46 *M. tuberculosis* strains by deletion analysis revealed that most RD regions were present in all *M. tuberculosis* strains tested (Table 2). Only regions RD3 and RD11, corresponding to the two prophages phiRv1 and phiRv2 of *M. tuberculosis* H37Rv (4), RD6 containing the insertion sequence IS*1532*, and RD5 that is flanked by a copy of IS*6110* (5) were absent in some strains. This is an important observation as it implies that *M. tuberculosis* strains are highly conserved with respect to RD1, RD2, RD4, RD7, RD8, RD9, RD10, RD12, RD13, and RD14, and that these RDs represent regions that can differentiate *M. tuberculosis* strains independent of their geographical origin and their typing characteristics from certain other members of the *M. tuberculosis* complex. Furthermore, this suggests that these regions may be involved in the host specificity of *M. tuberculosis*.

In contrast, the presence or absence of RvD regions in *M. tuberculosis* strains was

variable. The region which showed the greatest variability was RvD2, since 18 from 46 tested *M. tuberculosis* strains did not carry the RvD2 region. Strains with a high copy number of IS*6110* (>14) missed regions RvD2 to RvD5 more often than strains with only a few copies. As an example, all six tested strains belonging to the Beijing cluster (8) lacked regions RvD2 and RvD3. This is in agreement with the proposed involvement of recombination of two adjacent copies of IS*6110* in this deletion event (7).

However, the most surprising finding concerning the RvD regions was that TbD1 was absent from 40 of the tested *M. tuberculosis* strains (87 %), including representative strains from major epidemics such as the Haarlem, Beijing and Africa clusters (8). To accentuate this result we named this region "*M. tuberculosis* specific deletion 1" (TbD1). *In silico* sequence comparison of *M. tuberculosis* H37Rv with the corresponding section in *M. bovis* AF2122/97 revealed that in *M. bovis* this locus comprises two genes encoding membrane proteins belonging to a large family, whereas in *M. tuberculosis* H37Rv one of these genes (*mmpS6*) was absent and the second was truncated (*mmpL6*). Unlike the RvD2-RvD5 deletions, the TbD1 region is not flanked by a copy of IS*6110* in *M. tuberculosis* H37Rv, suggesting that insertion elements were not involved in the deletion of the 2153 bp fragment. To further investigate whether the 40 *M. tuberculosis* strains lacking the TbD1 region had the same genomic organization of this locus as *M. tuberculosis* H37Rv, we amplified the TbD1-junction regions of the various strains by PCR using primers flanking the deleted region (Table 1). This approach showed that the size of the amplicons obtained from multiple strains was uniform (Fig. 1) and subsequent sequence analysis of the PCR products revealed that in all tested TbD1-deleted strains the sequence of the junction regions was identical to that of *M. tuberculosis* H37Rv (Fig.2). The perfect conservation of the junction sequences in TbD1-deleted strains of wide geographical diversity suggests that the genetic event which resulted in the deletion occurred in a common progenitor. However, six *M. tuberculosis* strains, all characterized by very few or no copies of IS*6110* and spoligotypes that resembled each other (Fig. 3) still had the TbD1 region present. Interestingly, these six strains were also clustered together by MIRU-VNTR analysis (9).

Analysis of partial gene sequences of *oxyR, pncA, katG*, and *gyrA* which have been described as variable between different tubercle bacilli (2, 11, 12, 13) revealed that all tested *M. tuberculosis* strains showed *oxyR* and *pncA* partial sequences typical for *M. tuberculosis* (*oxyR* - nucleotide 285 ($oxyR^{285}$):G, *pncA* – codon 57 ($pncA^{57}$: CAC ). Based on the *katG* codon 463 ($katG^{463}$) and *gyrA* codon 95 ($gyrA^{95}$) sequence polymorphism, Sreevatsan and colleagues (2) defined three groups among the tubercle bacilli, group 1 showing $katG^{463}$

CTG (Leu), *gyrA*$^{95}$ ACC (Thr), group 2 exhibiting *katG*$^{463}$ CGG (Arg), *gyrA*$^{95}$ ACC (Thr), and group 3 showing *katG*$^{463}$ CGG (Arg), *gyrA*$^{95}$ AGC (Ser). According to this scheme, in our study 16 of the 46 tested *M. tuberculosis* strains belonged to group 1, whereas 27 strains belonged to group 2 and only 3 isolates to group 3. From the 40 strains that were deleted for region TbD1, 9 showed characteristics of group 1, including the strains belonging to the Beijing cluster, 28 of group 2, including the strains from the Haarlem and Africa clusters and 3 of group 3, including H37Rv and H37Ra. Most interestingly, all six *M. tuberculosis* strains where the TbD1 region was not deleted, contained a leucine (CTG) at *katG*$^{463}$, which was described as characteristic for ancestral *M. tuberculosis* strains (group 1) (2). As shown in Figure 4, this suggests that during the evolution of *M. tuberculosis* the *katG* mutation at codon 463 CTG (Leu) → CGG (Arg) occurred in a progenitor strain that had region TbD1 deleted. This proposal is supported by the finding that strains belonging to group 1 may or may not have deleted region TbD1, whereas all 30 strains belonging to groups 2 and 3 lacked TbD1 (Fig. 4). Furthermore, all strains of groups 2 and 3 characteristically lacked spacer sequences 33-36 in the direct repeat (DR) region (Fig. 3). It appears that such spacers may be lost but not gained (14). Therefore, TbD1 deleted strains will be referred to hereafter as "modern" *M. tuberculosis* strains.

**2.2. *M. canettii:***

*M. canettii* is a very rare smooth variant of *M. tuberculosis*, isolated usually from patients from, or with connection to, Africa. Although it shares identical 16S rRNA sequences with the other members of the *M. tuberculosis* complex, *M. canettii* strains differ in many respects including polymorphisms in certain house-keeping genes, IS*1081* copy number, colony morphology, and the lipid content of the cell wall (15, 16). Therefore, we were surprised to find that in *M. canettii* all the RD, RvD, and TbD1 regions except the prophages (phiRv1, phiRv2) were present. In contrast, we identified a region (RD$^{can}$) being specifically absent from all five *M. canettii* strains that partially overlapped RD12 (Fig. 4).

The conservation of the RD, RvD, and TbD1 regions in the genome of *M. canettii* in conjunction with the many described and observed differences suggest that *M. canettii* diverged from the common ancestor of the *M. tuberculosis* complex before RD, RvD and TbD1 occurred in the lineages of tubercle bacilli (Fig. 4). This hypothesis is supported by the finding that *M. canettii* was shown to carry 26 unique spacer sequences in the direct repeat region (14), that are no longer present in any other member of the *M. tuberculosis* complex. Therefore, *M. canettii* represents a fascinating tubercle bacillus, whose detailed genomic

analysis may reveal further insights into the evolution of the *M. tuberculosis* complex.

*2.3. M. africanum:*

The isolates designated as *M. africanum* studied here originate from West and East-African sources. 11 strains were isolated in Sierra Leone, Nigeria and Guinea and 2 strains in Uganda. One strain comes from the Netherlands.

For the 11 West African isolates, RD analysis indicated that these strains all lack the RD9 region containing *cobL*. Sequence analysis of the RD9 junction region showed that the genetic organization of this locus in West African strains was identical to that of *M. bovis* and *M. microti* in that the 5' part of *cobL* as well as the genes Rv2073c and Rv2074c were absent. In addition, six strains (2 from Sierra Leone, 4 from Guinea) also lacked RD7, RD8 and RD10 (Table 2). The junction sequences bordering RD7, RD8 and RD10, like those for RD9, were identical to those of *M. bovis* and *M. microti* strains. As regards the two prophages phiRv1 and phiRv2, the West African strains all contained phiRv2, whereas phiRv1 was absent. No variability was seen for the RvD regions. RvD1-RvD5 and TbD1 were present in all tested West African strains. This shows that *M. africanum* prevalent in West Africa can be differentiated from "modern" *M. tuberculosis* by at least two variable genetic markers, namely the absence of region RD9 and the presence of region TbD1.

In contrast, for East African *M. africanum* and for the isolate from the Netherlands, no genetic marker was found which could differentiate them from *M. tuberculosis* strains. With the exception of prophage phiRv1 (RD3) the 3 strains from Uganda and the Netherlands did not exhibit any of the RD deletions, but lacked the TbD1 region, as do "modern" *M. tuberculosis* strains. The absence of the TbD1 region was also confirmed by sequence analysis of the TbD1 junction region, which was found to be identical to that of TbD1 deleted *M. tuberculosis* strains. These results indicate a very close genetic relationship of these strains to *M. tuberculosis* and suggest that they should be regarded as *M. tuberculosis* rather than *M. africanum* strains.

*2.4. M. microti:*

*M. microti* strains were isolated in the 1930's from voles (17) and more recently from immuno-suppressed patients (18). These strains are characterized by an identical, characteristic spoligotype, but differ in their IS*6110* profiles. Both, the vole and the human isolates, lacked regions RD7, RD8, RD9, and RD10 as well as a region that is specifically deleted from *M. microti* ($RD^{mic}$). $RD^{mic}$ was revealed by a detailed comparative genomics

study of *M. microti* isolates (19) using clones from a *M. microti* Bacterial Artificial Chromosome (BAC) library. $RD^{mic}$ partially overlaps RD1 from BCG (data not shown). Furthermore, vole isolates missed part of the RD5 region, whereas this region was present in the human isolate. As the junction region of RD5 in *M. microti* was different to that in BCG (data not shown), RD5 was not used as an evolutionary marker.

*2.5. M. bovis* and *M. bovis* BCG:

*M. bovis* has a very large host spectrum infecting many mammalian species, including man. The collection of *M. bovis* strains that was screened for the RD and RvD regions consisted of 2 BCG strains and 18 "classical" *M. bovis* strains generally characterized by only one or two copies of IS*6110* from bovine, llama and human sources in addition to three goat isolates, three seal isolates, two oryx isolates, and two *M. bovis* strains from humans that presented a higher number of IS*6110* copies.

Excluding prophages, the distribution of RDs allowed us to differentiate five main groups among the tested *M. bovis* strains. The first group was formed by strains that lack RD7, RD8, RD9, and RD10. Representatives of this group are three seal isolates and two human isolates containing between three and five copies of IS*6110* (data not shown). Two oryx isolates harboring between 17 and 20 copies of IS*6110* formed the second group that lacked parts of RD5 in addition to RD7-RD10, and very closely resembled the *M. microti* isolates. However, they did not show $RD^{mic}$, the deletion characteristic of *M. microti* strains (data not shown). Analysis of partial *oxyR* and *pncA* sequences from strains belonging to groups one and two, showed sequence polymorphisms characteristic of *M. tuberculosis* strains ($oxyR^{285}$: G, $pncA^{57}$: CAC, Ref. 12, 13).

Group three consists of goat isolates that lack regions RD5, RD7, RD8, RD9, RD10, RD12, and RD13. As previously described by Aranaz and colleagues, these strains exhibited an adenosine at position 285 of the *oxyR* pseudogene that is specific for "classical" *M. bovis* strains whereas the sequence of the $pncA^{57}$ polymorphism was identical to that in *M. tuberculosis* (20). This is in good agreement with our results from sequence analysis (Table 2) and the finding that except for RD4, the goat isolates displayed the same deletions as "classical" *M. bovis* strains. Taken together, this suggests that the $oxyR^{285}$ mutation (G → A) occurred in *M. bovis* strains before RD4 was lost. Interestingly, the most common *M. bovis* strains ("classical" *M. bovis* (21)), isolated from cattle from Argentina, the Netherlands, the UK and Spain, as well as from humans (e. g. multi-drug resistant *M. bovis*

from Spain (22)) showed the greatest number of RD deletions and appear to have undergone the greatest loss of DNA relative to other members of the *M. tuberculosis* complex. These lacked regions RD4, RD5, RD6, RD7, RD8, RD9, RD10, RD12 and RD13, confirming results obtained with reference strains (5, 6). These strains together with the two BCG strains were the only ones that showed the $pncA^{57}$ polymorphism GAC (Asp) in addition to the $oxyR^{285}$ mutation (G → A) characteristic of *M. bovis*. Analysis of BCG strains indicate that BCG lacked the same RD regions as "classical" *M. bovis* strains in addition to RD1, RD2 and RD14 which apparently occurred during and after the attenuation process (Fig. 4) (6, 23).

In contrast to RDs, the RvD regions were highly conserved in the *M. bovis* strains. With the exception of the two IS*6110*-rich oryx isolates, that lacked RvD2, RvD3 and RvD4, all other strains had the five RvD regions present. It is particularly noteworthy that TbD1 was present in all *M. bovis* strains.

However, except for the two human isolates, containing between three and five copies of IS*6110* from group 1, strains designated as *M. bovis* showed a single nucleotide polymorphism in the TbD1 region at codon 551 (AAG) of the *mmpL6* gene, relative to *M. canettii*, *M. africanum* and ancestral *M. tuberculosis* strains, which are characterized by codon AAC. Even the strains isolated from seals and from oryx with *oxyR* or *pncA* loci like those of *M. tuberculosis* and with fewer deleted regions than the classical *M. bovis* strains, showed the $mmpL6^{551}$AAG polymorphism typical for *M. bovis* and *M. microti* (Table 2, Fig. 4). As such, this polymorphism could serve as a very useful genetic marker for the differentiation of strains that lack RD7, RD8, RD9, and RD10 and have been classified as *M. bovis* or *M. africanum*, but may differ from other strains of the same taxon.

## 3. DISCUSSION

### 3.1. Origin of human tuberculosis

For many years, it was thought that human tuberculosis evolved from the bovine disease by adaptation of an animal pathogen to the human host (3). This hypothesis is based on the property of *M. tuberculosis* to be almost exclusively a human pathogen, whereas *M. bovis* has a much broader host range. However, the results from this study unambiguously show that *M. bovis* has undergone numerous deletions relative to *M. tuberculosis*. This is confirmed by the preliminary analysis of the near complete genome sequence of *M. bovis* AF2122/97, a "classical" *M. bovis* strain isolated from cattle, which revealed no new gene

clusters that were confined specifically to *M. bovis*. This indicates that the genome of *M. bovis* is smaller than that of *M. tuberculosis* (24). It seems plausible that *M. bovis* is the final member of a separate lineage represented by *M. africanum* (RD9), *M. microti* (RD7, RD8, RD9, RD10) and *M. bovis* (RD4, RD5, RD7, RD8, RD9, RD10, RD12, RD13) (25) that branched from the progenitor of *M. tuberculosis* isolates. Successive loss of DNA may have contributed to clonal expansion and the appearance of more successful pathogens in certain new hosts.

Whether the progenitor of extant *M. tuberculosis* strains was already a human pathogen when the *M. africanum* → *M. bovis* lineage separated from the *M. tuberculosis* lineage is a subject for speculation. However, we have two reasons to believe that this was the case. Firstly, the six ancestral *M. tuberculosis* strains (TbD1$^+$, RD9$^+$) (Fig.3) that resemble the last common ancestor before the separation of *M. tuberculosis* and *M. africanum* are all human pathogens. Secondly, *M. canettii*, which probably diverged from the common ancestor of today's *M. tuberculosis* strains prior to any other known member of the *M. tuberculosis* complex is also a human pathogen. Taken together, this means that those tubercle bacilli, which are thought to most closely resemble the progenitor of *M. tuberculosis* are human and not animal pathogens. It is also intriguing that most of these strains were of African or Indian origin (Fig. 3). It is likely that these ancestral strains predominantly originated from endemic foci (15, 26), whereas "modern" *M. tuberculosis* strains that have lost TbD1 may represent epidemic *M. tuberculosis* strains that were introduced into the same geographical regions more recently as a consequence of the worldwide spread of the tuberculosis epidemic.

### 3.2. The evolutionary timescale of the *M. tuberculosis* complex

Because of the high sequence conservation in housekeeping genes, Sreevatsan *et al.* previously hypothesized that the tubercle bacilli encountered a major bottleneck 15,000 – 20,000 years ago (2). As the conservation of the TbD1 junction sequence in all tested TbD1 deleted strains suggests descendance from a single clone, the TbD1$^-$ deletion is a perfect indicator that "modern" *M. tuberculosis* strains that account for the vast majority of today's tuberculosis cases definitely underwent such a bottleneck and then spread around the world.

As described in detail in the results section, our analysis showed that the *katG*$^{463}$ CTG→CGG and the subsequent *gyrA*$^{95}$ ACC →AGC mutations, that were used by Sreevatsan and colleagues to designate groups 2 and 3 of their proposed evolutionary pathway of the tubercle bacilli (2), occurred in a lineage of *M. tuberculosis* strains that had

already lost TbD1 (Fig.4). Although deletions are more stable markers than point mutations, which may be subject to reversion, a perfect correlation of deletion and point mutation data was found for the tested strains.

This information, together with results from a recent study by Fletcher and colleagues (27), who have shown that *M. tuberculosis* DNAs amplified from naturally mummified Hungarian villagers from the 18th and 19th century belonged to $katG^{463}/gyrA^{95}$ groups 2 and 3, suggests that the TbD1 deletion occurred in the lineage of *M. tuberculosis* before the 18th century. This could mean that the dramatic increase of tuberculosis cases later in the 18th century in Europe mainly involved "modern" *M. tuberculosis* strains. In addition, it shows that tuberculosis was caused by *M. tuberculosis* and not by *M. bovis*, a fact which is also described for cases in rural medieval England (28).

There is good evidence that mycobacterial infections occurred in man several thousand years ago. We know that tuberculosis occurred in Egypt during the reign of the pharaohs because spinal and rib lesions pathognomonic of tuberculosis have been identified in mummies from that period (29). Identification of acid fast bacilli as well as PCR amplification of IS*6110* from Peruvian mummies (30) also suggest that tuberculosis existed in pre-Columbian societies of Central and South America. To estimate when the TbD1 bottleneck occurred, it would now be very interesting to know whether the Egyptian and South American mummies carried *M. tuberculosis* DNA that had TbD1 deleted or not.

The other major bottleneck, which seems to have occurred for members of the *M. africanum* → *M. microti* → *M. bovis* lineage is reflected by RD9 and the subsequent RD7, RD8 and RD10 deletions (Fig. 4). These deletions seem to have occurred in the progenitor of tubercle bacilli that - today - show natural host spectra as diverse as humans in Africa, voles on the Orkney Isles (UK), seals in Argentina, goats in Spain, and badgers in the UK. For this reason it is difficult to imagine that spread and adaptation of RD9-deleted bacteria to their specific hosts could have appeared within the postulated 15,000 – 20,000 years of speciation of the *M. tuberculosis* complex.

However, more insight into this matter could be gained by RD analysis of ancient DNA samples, e. g. mycobacterial DNA isolated from a 17,000 year old bison skeleton (31). The mycobacterium whose DNA was amplified showed a spoligotype that was most closely related to patterns of *M. africanum* and could have been an early representative of the lineage *M. africanum* →*M. bovis*. With the TbD1 and RD9 junction sequences that we supply here, PCR analyses of ancient DNAs should enable very focused studies to be undertaken to learn more about the timescale within which the members of the *M.*

*tuberculosis* complex have evolved.

**3.3. Concluding comments**

Our study provides an overview of the diversity and conservation of variable regions in a broad range of tubercle bacilli. Deletion analysis of 100 strains from various hosts and countries has identified some evolutionarily "old" *M. canettii, M. tuberculosis* and *M. africanum* strains, most of them of African origin, as well as "modern" *M. tuberculosis* strains, the latter including representatives from major epidemic clusters like Beijing, Haarlem and Africa. The use of deletion analysis in conjunction with molecular typing and analysis of specific mutations was shown to represent a very powerful approach for the study of the evolution of the tubercle bacilli and for the identification of evolutionary markers. In a more practical perspective, these regions, primarily RD9 and TbD1 but also RD1, RD2, RD4, RD7, RD8, RD10, RD12 and RD13 represent very interesting candidates for the development of powerful diagnostic tools for the rapid and unambiguous identification of members of the *M. tuberculosis* complex (32). This genetic approach for differentiation can now be used to replace the often confusing traditional division of the *M. tuberculosis* complex into rigidly defined subspecies.

Moreover, functional analyses will show whether the TbD1 deletion confers some selective advantage to "modern" *M. tuberculosis*, or whether other circumstances contributed to the pandemic of the TbD1 deleted *M. tuberculosis* strains.

**EXAMPLE 4**

The members of the *M. tuberculosis* complex share en unusually high degree of conservation such that the commercially-available nucleic acid probes and amplification assays cannot differentiate these organisms. In addition conventional identification methods are often ambiguous, cumbersome and time consuming because of the slow growth of the organisms.

In the present invention the inventors, by a deletion analysis, solve the problem faced by clinical mycobacteriology laboratories for differentiation within the *M. tuberculosis* complex.

This approach allows to perform a diagnostic on a biological fluid by using at least three markers including TBD1. The following table 3 illustrates such a combinaison sufficient to realize the distinction between the members of the *M. tuberculosis* complex.

| | MARKERS | | |
|---|---|---|---|
| **MYCOBACTERIUM STRAIN** | **RD4** | **RD9** | **TBD1** |
| M. bovis BCG | - | - | + |
| M. bovis | - | - | + |
| M. africanum | + | - | + |
| M. tuberculosis | + | + | - |
| M. tuberculosis ancêtre | + | + | + |
| M. canettii | + | + | + |

**Table 3**

Beside TbD1 marker, preferably at least 2 other markers should be used. Examples of such additional markers available in the literature are listed in the following table 1.

**Supplemental data:**

**Table 1:** RD, RvD and TbD1 regions and selected primers

| Region absent from BCG | Gene | Size (kb) | Internal Primerpair | Flanking primers or 2nd internal * primerpair |
|---|---|---|---|---|
| RD1 | Rv3871-Rv3879c | 9.5 | RD1in-Rv3878F<br>GTC AGC CAA GTC AGG CTA CC<br>RD1in-Rv3878R<br>CAA CGT TGT GGT TGT TGA GG | RD1-flank.left<br>GAA ACA GTC CCC AGC AGG T<br>RD1-flank.right<br>TTC AAC GGG TTA CTG CGA AT |
| RD2 | Rv1978-Rv1988 | 10.8 | RD2-Rv1979.int.F<br>TAT AGC TCT CGG CAG GTT CC<br>RD2-Rv1979-int.R<br>ATC GGC ATC TAT GTC GGT GT | RD2-flank.F<br>CTC GAC CGC GAC GAT GTG C<br>RD2-flank.R<br>CCT CGT TGT CAC CGC GTA TG |
| RD3* | Rv1573-Rv1586c | 9.2 | RD3-Rv1586.int.F<br>TTA TCT TGG CGT TGA CGA TG<br>RD3-Rv1586.int.R<br>CAT ATA AGG GTG CCC GCT AC | RD3-int-REP.F<br>CTG ACG TCG TTG TCG AGG TA*<br>RD3-int-REP.R<br>GTA CCC CCA GGC GAT CTT* |
| RD4 | Rv1505c-Rv1516c | 12.7 | RD4-Rv1516.int.F<br>CAA GGG GTA TGA GGT TCA CG<br>RD4-Rv1516.int.R<br>CGG TGA TTC GTG ATT GAA CA | RD4-flank.F<br>CTC GTC GAA GGC CAC TAA AG<br>RD4-flank.R<br>AAG GCG AAC AGA TTC AGC AT |
| RD5* | Rv2346c-Rv2353c | 9.0 | RD5A-Rv2348.int.F<br>AAT CAC GCT GCT GCT ACT CC<br>RD5A-Rv2348.int.R<br>GTG CTT TTG CCT CTT GGT C | RD5B-plcA.int.F<br>CAA GTT GGG TCT GGT CGA AT<br>RD5B-plcA.int.R<br>GCT ACC CAA GGT CTC CTG GT |
| RD6* | Rv3425-Rv3428c | 4.9 | RD6-IS1532F<br>CAG CTG GTG AGT TCA AAT GC<br>RD6-IS1532R<br>CTC CCG ACA CCT GTT CGT | ND<br><br>ND |
| RD7 | Rv1964-Rv1977 | 12.7 | RD7-Rv1976.int.F<br>TGG ATT GTC GAC GGT ATG AA<br>RD7-Rv1976.int.R<br>GGT CGA TAA GGT CAC GGA AC | RD7-flank.F<br>GGT AAT CGT GGC CGA CAA G<br>RD7-flank.R<br>CAG CTC TTC CCC TCT CGA C |
| RD8 | *ephA-lpqG* | 5.9 | RD8-ephA.F<br>GGT GTG ATT TGG TGA GAC GAT G | RD8-flank.F<br>CAA TCA GGG CTG TGC TAA CC |

| | | | | |
|---|---|---|---|---|
| | | | RD8-ephA.R | RD8-flank.R |
| | | | AGT TCC TCC TGA CTA ATC CAG GC | CGA CAG TTG TGC GTA CTG GT |
| RD9 | *cobL*-Rv2075 | 2.0 | RD9-intF | RD9-flankF |
| | | | CGA TGG TCA ACA CCA CTA CG | GTG TAG GTC AGC CCC ATC C |
| | | | RD9-intR | RD9-flankR |
| | | | CTG GAC CTC GAT GAC CAC TC | GCC CAA CAG CTC GAC ATC |
| RD10 | Rv0221-Rv0223 | 1.9 | RD10-intF | RD10-flankF |
| | | | GTA ACC GCT TCA CCG GAA T | CTG CAA CCA TCC GGT ACA C |
| | | | RD10-intR | RD10-flankR |
| | | | GTC AAC TCC ACG GAA AGA CC | GTC ATG AAC GCC GGA CAG |
| RD11 | Rv2645-Rv2695c | 11.0 | RD11-Rv2646F | RD11-fla-F |
| | | | CGG CAG CTA GAC GAC CTC | TCA CAT AGG GGC TGC GAT AG |
| | | | RD11-Rv2646R | RD11-fla-R |
| | | | AAC GTG CTG CGA TAG GTT TT | AGA GGA ACC TTT CGG TGG TT |
| RD12 | *sseC*-Rv3121 | 2.8 | RD12-Rv3120.int.F | RD12-flank.F |
| | | | GAA ATA CGA GTG CGC TGA CC | GCC ATC AAC GTC AAG AAC CT |
| | | | RD12-Rv3120.int.R | RD12-flank.R |
| | | | CTC TGA ACC ATC GGT GTC G | CGG CCA GGT AAC AAG GAG T |
| RD13 | Rv1255c-Rv1257c | 3.0 | RD13intF | RD13-flank.F |
| | | | GGA TGT CAC TCG GAA CGG CA | CGA TGG TGT TTC TTG GTG AG |
| | | | RD13intR | RD13-flank.R |
| | | | CAC CGG GCT GAT CGA GCG A | GGA TCG GCT CAG TGA ATA CC |
| RD14 | Rv1765c-Rv1773c | 9.0 | RD14-Rv1769.int.F | RD14-flankF |
| | | | GTG GAG CAC CTT GAC CTG AT | TTG ATT CGC CAA CAA CTG AA |
| | | | RD14-Rv1769.int.R | RD14-flankR |
| | | | CGT CGA ATA CGA GTC GAA CA | GGG CTG GTT AGT GTC GAT TC |
| **Region missing from *M. tuberculosis* H37Rv** | | | | |
| | | | RvD1-int1F | RvD1-int2.F |
| RvD1* | | 5.0 | AGC GCG TCG AAC ACC GGC | GAG CCA CTC CGA TGT TGA CT |
| | | | RvD1-int1R | RvD1-int2.R |
| | | | CCT GAA TCC GCG CAA TTC CAT | CAC GCG AAC CCT ACC TAC AT |
| | | | RvD2-int1F | RvD2-int2F |
| RvD2* | *plcD* | 5.1 | GTT CTC CTG TCG AAC CTC CA | GGA CGG TGA CGG TAT TTG TC |
| | | | RvD2-int1R | RvD2-int2R |
| | | | ACT TCA CCG GTT TCA TCT CG | TCG CCA ACT TCT ATG GAC CT |
| | | | RvD3-intF | RvD3-flank.F |
| RvD3 | | 1.0 | ATC GAT CAG GTC GTC AAT GC | AAA CCA TGC AGC GTC TGC CA |
| | | | RvD3-intR | RvD3-flankR |
| | | | ACG CCA CCA TCA AGA TCC | GCG TTT CTG CGT CTG GTT GA |

| | | | | |
|---|---|---|---|---|
| RvD4* | PPE gene | 0.8 | RvD4-intF-PPE<br>GGT TGC CAA CGT TAC CGA TGC | ND |
| | | | RvD4-intR-PPE<br>CCG GTG GTG GTG GCG GCT | ND |
| RvD5 | *moa* | 4.0 | RvD5intF<br>GGG TTC ACG TTC ATT ACT GTT C | RvD5-flankF<br>CCC ATC GTG GTC GTT CAC C |
| | | | RvD5intR<br>CCT GCG CTT ATC TCT AGC GG | RvD5-flankR<br>GTA CCC GCA CCA CCT GCT G |
| TbD1 | *mmpL6* | 2.1 | TBD1intS.F<br>CGT TCA ACC CCA AAC AGG TA | TBD1fla1-F<br>CTA CCT CAT CTT CCG GTC CA |
| | | | TBD1intS.R<br>AAT CGA ACT CGT GGA ACA CC | TBD1fla1-R<br>CAT AGA TCC CGG ACA TGG TG |
| ***katG, gyrA, oxyR', pncA* and *mmpL6* PCR and sequencing primers** | | | | |
| *katG*$^{463}$ | | | *katG*-2154,225-PCR-F<br>CTA CCA GCA CCG TCA TCT CA | *katG*-2154,872-SEQ-R<br>ACA AGC TGA TCC ACC GAG AC |
| | | | *katG*-2155,157-PCR-R<br>AGG TCG TAT GGA CGAACA CC | |
| *gyrA*$^{95}$ | | | *gyrA*-7,127-PCR-F<br>GTT CGT GTG TTG CGT CAA GT | *gyrA*-7,461F<br>CGG GTG CTC TAT GCA ATG TT |
| | | | *gyrA*- 8,312-PCR-R<br>CAG CTG GGT GTG CTT GTA AA | |
| *oxyR*$^{i285}$ | | | *oxyR* 2725,559F<br>TAT GCG ATC AGG CGT ACT TG | *oxyR*-2726,024-SEQ-R<br>CAA AGC AGT GGT TCA GCA GT |
| | | | *oxyR*-2726,024-PCR-R<br>CAA AGC AGT GGT TCA GCA GT | |
| *pncA*$^{57}$ | | | *pncA*–2288,678-PCR-F<br>ATC AGG AGC TGC AAA CCA AC | *pncA*- 2289,319-SEQ-R<br>GGC GTC ATG GAC CCT ATA TC |
| | | | *pncA*- 2289,319-PCR-R<br>GGC GTC ATG GAC CCT ATA TC | |
| *mmpL6*$^{551}$ | | | *mmpL*-seq5F<br>GTA TCA GAG GGA CCG AGC AG | *mmpL*-seq5F<br>GTA TCA GAG GGA CCG AGC AG |
| | | | TBD1fla1-R<br>CAT AGA TCC CGG ACA TGG TG | |

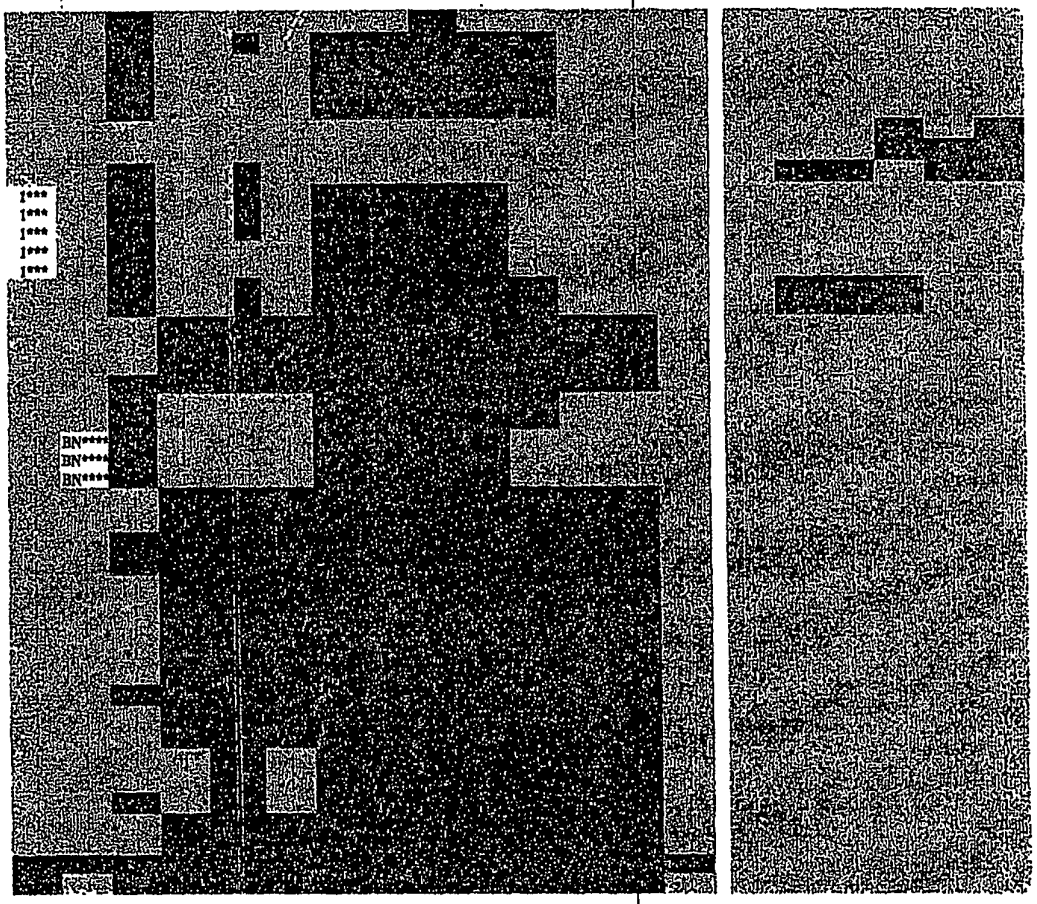
The RD nomenclature used in this table is based on that used by Brosch *et al.* (2000), (Ref. 25) and differs from that proposed by Behr and coworkers (1999), (Ref. 6). Primer sequences are shown in 5' →3' direction.

* Regions where a second pair of internal primers was used rather than flanking primers, due to flanking repetitive regions, and/or mobile genetic elements.

Web-Table 2

| Ident. No. | Isolate No. | Species | Host | Country | *katG* | *gyrA* | *oxyR* | *pncA* | *rrnpL6* | RD1 | RD2 | RD3 | RD4 | RD5a,b | RD6 | RD7 | RD8 | RD9 | RD10 | RD11 | RD12 | RD13 | RD14 | RvD1 | RvD2 | RvD3 | RvD4 | RvD5 | TbD1 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| (from Ref. 8) | 10059 | *M.canettii* | human | France | T | C | G | C | C | | | | | | | | | | | | BN***** | | | | | | | | |
| | 10060 | *M.cas.ettu* | human | France | T | C | G | C | C | | | | | | | | | | | | BN***** | | | | | | | | |
| | 10061 | *M.canettii* | human | Papeete | T | C | G | C | C | | | | | | | | | | | | BN***** | | | | | | | | |
| | 981514 | *M. canettii* | human | Djibouti | T | C | G | C | C | | | nd | | | | | | | | | BN***** | | | | | | | | |
| 129 | 48 | *M. canetti* | human | Somalia | T | C | G | C | C | | | | | | | | | | | | | | | | | | | | |
| 131 (7) | 1 (114) | *M. tuberculosis* | human | Mongolia | G | C | G | C | * | | | | | | | | | | | | | | | | | | | | |
| 103 (108) | 2 (115) | *M. tuberculosis* | human | China | G | C | G | C | * | | | | | | | | | | | | | | | | | | | | |
| 68 | 3 | *M. tuberculosis* | H37Rv | USA | G | G | G | C | * | | | | | | | | | | | | | | | | | | | | |
| 22 (20) | 5 (117) | *M. tuberculosis* | human | Mongolia | T | C | G | C | * | | | | | | | | | | | | | | | | | | | | |
| 84 (50) | 6 (118) | *M. tuberculosis* | human | Czech Rep. | G | C | | | * | | | | | | | | | | | | | | | | | | | | |
| 112 | 7 | *M. tuberculosis* | human | Netherlands | T | C | G | C | * | | | | | | | | | | | | | | | | | | | | |
| 124 (98) | 8 (119) | *M. tuberculosis* | human | Equator | G | C | G | C | * | | | | | | | | | | | | | | | | | | | | |
| 104 (27) | 9 (120) | *M. tuberculosis* | human | Ethiopia | G | C | | C | * | | | | | | | | | | | | | | | | | | | | |
| 43 | 10 | *M. tuberculosis* | human | China | T | C | | | * | | | | | | | | | | | | | | | | | | | | |
| 15 (55) | 11 (121) | *M. tuberculosis* | human | Iran | G | C | G | C | * | | | | | | | | | | | | | | | | | | | | |
| 60 | 12 | *M. tuberculosis* | human | Bolivia | G | C | G | | * | | | | | | | | | | | | | | | | | | | | |
| 86 | 13 | *M. tuberculosis* | human | Bolivia | G | C | | | * | | | | | | | | | | | | | | | | | | | | |
| 109 | 14 | *M. tuberculosis* | H37Ra | USA | G | G | | | * | | | | | | | | | | | | | | | | | | | | |
| 120 | 16 | *M. tuberculosis* | human | Burundi | G | C | G | C | * | | | | | | | | | | | | | | | | | | | | |
| 40 | 17 | *M. tuberculosis* | human | Burundi | G | C | | | * | | | | | | | | | | | | | | | | | | | | |
| 82 | 18 | *M. tuberculosis* | human | Canada | G | C | | | * | | | | | | | | | | | | | | | | | | | | |
| 46 | 23 | *M. tuberculosis* | human | Chili | G | C | | | * | | | | | | | | | | | | | | | | | | | | |
| 59 (10) | 25 (123) | *M. tuberculosis* | human | Equator | G | C | G | C | * | | | | | | | | | | | | | | | | | | | | |
| 118 | 26 | *M. tuberculosis* | human | Honduras | G | C | | | * | | | | | | | | | | | | | | | | | | | | |
| 74 (102) | 28 (125) | *M. tuberculosis* | human | India | T | C | G | C | C | | | | | | | | | | | | | | | | | | | | |
| 19 | 29 | *M. tuberculosis* | human | India | T | C | | | * | | | | | | | | | | | | | | | | | | | | |
| 36 | 30 | *M. tuberculosis* | human | India | T | C | | | C | | | | | | | | | | | | | | | | | | | | |
| 99 (63) | 32 (98) | *M. tuberculosis* | human | Italy | G | C | | | * | | | | | | | | | | | | | | | | | | | | |
| 94 (37) | 33 (106) | *M. tuberculosis* | human | Uganda | G | C | | | * | | | | | | | | | | | | | | | | | | | | |
| 97 | 34 | *M. tuberculosis* | human | Uganda | G | C | G | C | * | | | | | | | | | | | | | | | | | | | | |
| 79 (35) | 35 (107) | *M. tuberculosis* | human | Rwanda | G | C | G | C | * | | | | | | | | | | | | | | | | | | | | |
| 88 | 38 | *M. tuberculosis* | human | Russia | G | C | | | * | | | | | | | | | | | | | | | | | | | | |
| 114 (28) | 43 (111) | *M. tuberculosis* | human | Sri Lanka | G | C | G | C | * | | | | | | | | | | | | | | | | | | | | |
| 53 (61) | 44 | *M. tuberculosis* | human | Argentina | G | C | | | * | | | | | | | | | | | | | | | | | | | | |
| 21 | 46 | *M. tuberculosis* | human | Zimbabwe | T | C | | | C | | | | | | | | | | | | | | | | | | | | |
| 33 | 50 | *M. tuberculosis* | human | Netherlands | G | C | G | C | * | | | | | | | | | | | | | | | | | | | | |
| 56 | 79 | *M. tuberculosis* | human | Curacao | G | C | | | * | | | 1** | | | | | | | | | | | | | | | | | |
| 17 | 80 | *M. tuberculosis* | human | Greenland | G | C | G | C | * | | | | | | | | | | | | | | | | | | | | |
| 30 | 83 | *M. tuberculosis* | human | South Africa | T | C | G | C | * | | | | | | | | | | | | | | | | | | | | |
| 38 (75) | 87 (133) | *M. tuberculosis* | human | Tahiti | G | C | | | * | | | | | | | | | | | | | | | | | | | | |
| 49 | 88 | *M. tuberculosis* | human | Tanzania | T | C | | | * | | | | | | | | | | | | | | | | | | | | |
| 93 | 89 | *M. tuberculosis* | human | Tanzania | T | C | | | C | | | | | | | | | | | | | | | | | | | | |
| 90 | 94 | *M. tuberculosis* | human | South Korea | T | C | | | * | | | | | | | | | | | | | | | | | | | | |
| 34 | 95 | *M. tuberculosis* | human | Malaysia | T | C | | | * | | | | | | | | | | | | | | | | | | | | |
| 105 (96) | 102 (104) | *M. tuberculosis* | human | Netherlands | G | G | | | * | | | | | | | | | | | | | | | | | | | | |
| 67 | 103 | *M. tuberculosis* | human | Comoro Isl. | T | C | G | C | C | | | | | | | | | | | | | | | | | | | | |
| 65 (122) | 113 (53) | *M. tuberculosis* | human | Netherlands | T | C | | | C | | | | | | | | | | | | | | | | | | | | |
| 41 | 122 | *M. tuberculosis* | human | Chili | G | C | | | * | | | | | | | | | | | | | | | | | | | | |
| 11 | 127 | *M. tuberculosis* | human | Greenland | G | C | | | * | | | | | | | | | | | | | | | | | | | | |
| | Skin-Isolate | *M. tuberculosis* | human | Australia | T | C | G | C | * | | | | | | | | | | | | | | | | | | | | |
| | CDC1551 | *M. tuberculosis* | human | USA | G | C | G | C | * | | | | | | | | | | | | | | | | | | | | |
| | IS960090 | *M. africanum* 1 | human | SierraLeone | T | | G | C | C | | | | | | | | | | | | | | | | | | | | |
| | IS960092 | *M. africanum* 3 | human | SierraLeone | T | | | C | C | | | | | | | | | | | | | | | | | | | | |
| | IS960093 | *M. africanum* 4 | human | SierraLeone | T | | G | | C | | | | | | | | | | | | | | | | | | | | |
| | IS960094 | *M. africanum* 5 | human | SierraLeone | T | C | | | C | | | | | | | | | | | | | | | | | | | | |
| | IS960095 | *M. africanum* 6 | human | Nigeria | T | | G | | C | | | | | | | | | | | | | | | | | | | | |
| | IS960098 | *M. africanum* 9 | human | Nigeria | T | | | | C | | | | | | | | | | | | | | | | | | | | |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | IS960099 | *M. africanum* 10 | human | Nigeria | T | | | | C | |
| | IS960097 | *M. africanum* 8 | human | Guinea | T | | | | C | |
| | IS960102 | *M. africanum* 13 | human | Guinea | T | | G | | C | |
| | IS960103 | *M. africanum* 14 | human | Guinea | T | | | | C | |
| | IS960105 | *M. africanum* 16 | human | Guinea | T | | | | C | |
| | IS960096 | *"M. africanum"* 7 | human | Uganda | G | | G | | * | |
| | IS960109 | *"M. africanum"* 20 | human | Uganda | G | | | | * | |
| 100 | 55 | *"M. africanum"* | human | Netherlands | T | C | G | C | * | |
| 62 | 56 | *M. microti* | vole | UK | T | C | G | C | G | 1*** |
| 25 | 57 | *M. microti* | vole | UK | T | C | G | C | G | 1*** |
| | OV 254 | *M. microti* | vole | UK | T | | G | C | G | 1*** |
| | ATCC 35782 | *M. microti* | nd | nd | T | | G | C | G | 1*** |
| | 94/2272 | *M. microti* | human | Netherlands | T | | G | C | G | 1*** |
| 24 | 60 | *M. bovis* | oryx | Saoudi Arabia | T | C | G | C | G | |
| 69 | 61 | *M. bovis* | oryx | Netherlands | T | | G | C | G | |
| 117 | 62 | *M. bovis* | cattle | Argentinia | T | C | A | G | G | |
| 126 | 63 | *M. bovis* | cattle | Argentinia | T | | A | G | G | |
| 130 | 64 | *M. bovis* | cattle | Netherlands | T | | A | G | G | |
| 73 | 65 | *M. bovis* | cattle | Netherlands | T | C | A | G | G | |
| 85 (128) | 67 (131) | *"M. bovis"* | human | Netherlands | T | C | G | C | C | |
| 6 | 68 | *"M. bovis"* | human | Netherlands | T | C | G | C | C | |
| 81 | 39 | *M. bovis* | seal | Argentinia | T | | G | C | G | BN**** |
| 76 | 40 | *M. bovis* | seal | Argentinia | T | C | G | C | G | BN**** |
| 101 | 41 | *M. bovis* | seal | Argentinia | T | C | G | C | G | BN**** |
| | UZ 1 | *M. bovis* | llama | Spain | T | C | A | G | G | |
| | UZ 2 | *M. bovis* | human | Spain | T | | A | G | G | |
| | UZ 3 | *M. bovis* | human | Spain | T | | A | G | G | |
| | UZ 4 | *M. bovis* | human | Spain | T | | A | G | G | |
| | UZ 5 | *M. bovis* | human | Spain | T | | A | G | G | |
| | UZ 6 | *M. bovis* | cattle | Spain | T | | A | G | G | |
| | UZ 7 | *M. bovis* | human | Spain | T | C | A | G | G | |
| | UZ 8 | *M. bovis* | human | Spain | T | C | A | G | G | |
| | UZ 9 | *M. bovis* | cattle | Spain | T | C | A | G | G | |
| | UZ 10 | *M. bovis* | human | Spain | T | C | A | G | G | |
| | UZ 11 | *M. bovis* | human | Spain | T | | A | G | G | |
| | UZ 12 | *M. bovis* | human | Spain | T | | A | G | G | |
| | UZ 22 | *M. bovis* | goat | Spain | T | C | A | C | G | |
| | UZ 26 | *M. bovis* | goat | Spain | T | C | A | C | G | |
| | UZ 27 | *M. bovis* | goat | Spain | T | C | A | C | G | |
| | UZ 28 | *M. bovis* | human | Spain | T | C | A | G | G | |
| | AF2122/97 | *M. bovis* | cattle | UK | T | C | A | G | G | |
| 48 (2) | 4 (116) | *M. bovis* BCG | vaccine | France | T | | A | G | G | |
| 71 | 59 | *M. bovis* BCG | vaccine | Japan | T | C | A | G | G | |



0 = region not present (blue background)
1 = region present (yellow background)
* = not present
** = region partially deleted
*** = Rd mic
**** = Rd seal
***** = Rd can
BN = both negative
nd = not determined

Table 2: Strain identification numbers used in Ref. 8 and 9, geographical origin and information on the host, deletion and sequence characteristics of the100 strains belonging to the M. tuberculosis complex.

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# CLAIMS

1. An isolated or purified nucleic acid wherein said nucleic acid is selected from the group consisting of:

   a. SEQ ID N°1;

   b. Nucleic acid having a sequence fully complementary to SEQ ID N°1.

   c. Nucleic acid fragment comprising at least 15 consecutive nucleotides of SEQ ID N°1;

   d. Nucleic acid having at least 90% sequence identity after optimal alignment with a sequence defined in a) or b);

   e. Nucleic acid that hybridizes under stringent conditions with the nucleic acid defined in a) or b);

2. A nucleic acid fragment according to claim 1 wherein said nucleic acid fragment is:

   - specifically deleted from the genome of *Mycobacterium tuberculosis,* excepted in *Mycobacterium tuberculosis* strain having the mutation CTG -> CGG at codon 463 of gene katG and/or *Mycobacterium tuberculosis* strain having no or very few IS6110 sequences inserted in their genome; and,

   - present in the genome of *Mycobacterium africanum, Mycobacterium canetti, Mycobacterium microti, Mycobacterium bovis, Mycobacterium bovis BCG.*

3. A nucleic acid fragment according to claim 1 or 2 selected from the group consisting of:

   a) SEQ ID N°4;

   b) Nucleic acid having a sequence fully complementary to SEQ ID N°4.

   c) Nucleic acid fragment comprising at least 20 consecutive nucleotides of SEQ ID N°4;

   d) Nucleic acid having at least 90% sequence identity after optimal alignment with a sequence defined in a) or b);

   e) Nucleic acid that hybridizes under stringent conditions with the nucleic acid defined in a) or b);

4. The isolated or purified nucleic acid of claim 1 wherein said nucleic acid comprises at least a deletion of a nucleic acid fragment according to claim 2 or 3.

5. Isolated or purified polypeptides encoded by the nucleic acid of claims 1 to 4.

6. The polypeptide of claim 5 selected among polypeptide with sequence SEQ ID N° 5, N° 7, N° 9, N°11, and their fragments thereof.

7. Isolated or purified nucleic acid encoding the polypeptide according to claim 6.

8. Isolated or purified nucleic acid according to claim 7 selected among:
- SEQ ID N° 4 encoding the polypeptide of SEQ ID N°5;
- SEQ ID N° 6 encoding the polypeptide of SEQ ID N°7;
- SEQ ID N° 8 encoding the polypeptide of SEQ ID N°9;
- SEQ ID N° 10 encoding the polypeptide of SEQ ID N°11;

and their fragments thereof.

9. A recombinant vector comprising a nucleic acids sequence selected among nucleic acids according to claims 1 to 4 , 7 and 8.

10. The recombinant vector of claim 9 consisting of vector named X229 introduced into the recombinant *Escherichia coli* deposited with the CNCM on February 18th, 2002 under N° I-2799.

11. A recombinant cell comprising a nucleic acids sequence selected among nucleic acids according to claims 1 to 4 , 7 and 8, or a vector according to claims 9 and 10.

12. The recombinant cell according to claim 11 consisting of the *Escherichia coli* deposited with the CNCM on February 18th, 2002 under N° I-2799.

13. A method for the discriminatory detection and identification of:
- *Mycobacterium tuberculosis* excepted *Mycobacterium tuberculosis* strain having the mutation CTG -> CGG at codon 463 of gene katG and/or excepted *Mycobacterium tuberculosis* strain having no or very few IS6110 sequences inserted in their genome; versus,

- *Mycobacterium africanum, Mycobacterium canetti, Mycobacterium microti, Mycobacterium bovis, Mycobacterium bovis BCG* in a biological sample,

comprising the following steps:

a) isolation of the DNA from the biological sample to be analyzed or production of a cDNA from the RNA of the biological sample,

b) detection of the nucleic acid sequences of the mycobacterium present in said biological sample,

c) analysis for the presence or the absence of a nucleic acid fragment according to claims 2 or 3.

14. The method as claimed in claim 13, in which the detection of the mycobacterial DNA sequences is carried out using nucleotide sequences complementary to said DNA sequences.

15. The method as claimed in claim 13 or 14, in which the detection of the mycobacterial DNA sequences is carried out by amplification of these sequences using primers.

16. The method as claimed in claim 15, in which the primers have a nucleotide sequence chosen from the group comprising SEQ ID N°13, SEQ ID N°14, SEQ ID N°15, SEQ ID N°16, SEQ ID N°17, SEQ ID N°18.

17. A method for the discriminatory detection and identification of:

- *Mycobacterium tuberculosis* excepted *Mycobacterium tuberculosis* strain having the mutation CTG -> CGG at codon 463 of gene katG and/or excepted *Mycobacterium tuberculosis* strain having no or very few IS6110 sequences inserted in their genome; versus,

- *Mycobacterium africanum, Mycobacterium canetti, Mycobacterium microti, Mycobacterium bovis, Mycobacterium bovis BCG* in a biological sample,

comprising the following steps:

a) bringing the biological sample to be analyzed into contact with at least one pair of primers as defined in claim 15 or 16, the DNA contained in the sample having been, where appropriate, made accessible to the hybridization beforehand,

b) amplification of the DNA of the mycobacterium,

c) visualization of the amplification of the DNA fragments.

18. A kit for the discriminatory detection and identification of:

- *Mycobacterium tuberculosis* excepted *Mycobacterium tuberculosis* strain having the mutation CTG -> CGG at codon 463 of gene katG and/or excepted *Mycobacterium tuberculosis* strain having no or very few IS6110 sequences inserted in their genome; versus,
- *Mycobacterium africanum, Mycobacterium canetti, Mycobacterium microti, Mycobacterium bovis, Mycobacterium bovis BCG* in a biological sample,

in a biological sample comprising the following elements:

a) at least one pair of primers as defined in claim 15 or 16,

b) the reagents necessary to carry out a DNA amplification reaction,

c) optionally, the necessary components which make it possible to verify or compare the sequence and/or the size of the amplified fragment.

19. The use of at least one pair of primers as defined in claim 15 or 16 for the amplification of a DNA sequence from *Mycobacterium tuberculosis* or *Mycobacterium africanum, Mycobacterium canetti, Mycobacterium microti, Mycobacterium bovis, Mycobacterium bovis BCG, Mycobacterium tuberculosis* having the mutation CTG -> CGG at codon 463 of gene katG and/or having no or very few IS6110 sequences inserted in their genome.

20. A product of expression of all or part of the nucleic acid fragment as defined in claim 2 or 3 deleted from the genome of *Mycobacterium tuberculosis* and present in *Mycobacterium africanum, Mycobacterium canetti, Mycobacterium microti, Mycobacterium bovis, Mycobacterium bovis BCG, Mycobacterium tuberculosis* having the mutation CTG -> CGG at codon 463 of gene katG and/or having no or very few IS6110 sequences inserted in their genome; or conversely.

21. A method for the discriminatory detection *in vitro* of antibodies directed against *Mycobacterium tuberculosis* or *Mycobacterium africanum, Mycobacterium canetti, Mycobacterium microti, Mycobacterium bovis, Mycobacterium bovis BCG, Mycobacterium Tuberculosis* having the mutation CTG -> CGG at codon 463 of gene KatG and/or having no or very few IS6110 sequences inserted in their genome, in a biological sample, comprising the following steps:

a) bringing the biological sample into contact with at least one product as defined in claim 20,

b) detecting the antigen-antibody complex formed.

22. A method for the discriminatory detection of a vaccination with *Mycobacterium bovis* BCG or an infection by *Mycobacterium tuberculosis,* excepted *Mycobacterium Tuberculosis* strain having the mutation CTG -> CGG at codon 463 of gene KatG and/or excepted *Mycobacterium Tuberculosis* strain having no or very few IS6110 sequences inserted in their genome in a mammal, comprising the following steps:

a) preparation of a biological sample containing cells, more particularly cells of the immune system of said mammal and more particularly T cells,

b) incubation of the biological sample of step a) with at least one product as defined in claim 20,

c) detection of a cellular reaction indicating prior sensitization of the mammal to said product, in particular cell proliferation and/or synthesis of proteins such as gamma-interferon.

23. A kit for the *in vitro* diagnosis of *Mycobacterium tuberculosis* infection, excepted infection with *Mycobacterium tuberculosis* strain having the mutation CTG -> CGG at codon 463 of gene katG and/or having no or very few IS6110 sequences inserted in their genome, in a mammal optionally vaccinated beforehand with *M. bovis* BCG comprising:

a) a product as defined in claim 20,

b) where appropriate, the reagents for the constitution of the medium suitable for the immunological reaction,

c) the reagents allowing the detection of the antigen-antibody complexes produced by the immunological reaction,

d) where appropriate, a reference biological sample (negative control) free of antibodies recognized by said product,

e) where appropriate, a reference biological sample (positive control) containing a predetermined quantity of antibodies recognized by said product.

24. A mono- or polyclonal antibody, its chimeric fragments or antibodies, characterized in that they are capable of specifically recognizing a product as defined in claim 20.

25. A method for the discriminatory detection of the presence of an antigen of *Mycobacterium tuberculosis* or *Mycobacterium africanum, Mycobacterium canetti, Mycobacterium microti, Mycobacterium bovis, Mycobacterium bovis BCG, Mycobacterium*

*tuberculosis* having the mutation CTG -> CGG at codon 463 of gene katG and/or having no or very few IS6110 sequences inserted in their genome in a biological sample comprising the following steps:

a) bringing the biological sample into contact with an antibody as claimed in claim 24,

b) detecting the antigen-antibody complex formed.

26. A kit for the discriminatory detection of the presence of an antigen of *Mycobacterium tuberculosis* or *Mycobacterium africanum, Mycobacterium canetti, Mycobacterium microti, Mycobacterium bovis, Mycobacterium bovis BCG, Mycobacterium tuberculosis* having the mutation CTG -> CGG at codon 463 of gene katG and/or having no or very few IS6110 sequences inserted in their genome, in a biological sample comprising the following steps:

a) an antibody as claimed in claim 24,

b) the reagents for constituting the medium suitable for the immunological reaction,

c) the reagents allowing the detection of the antigen-antibody complexes produced by the immunological reaction.

27. An immunological composition, characterized in that it comprises at least one product as defined in claim 20.

28. A vaccine, characterized in that it comprises at least one product as defined in claim 20 in combination with a pharmaceutically compatible vehicle and, where appropriate, one or more appropriate immunity adjuvants.

29. An in vitro method for the detection and identification of *Mycobacterium tuberculosis* excepted *Mycobacterium tuberculosis* strain having the mutation CTG=> CGG at codon 463 of gene katG and/or excepted *Mycobacterium tuberculosis* strain having no or very few IS6110 sequences inserted in their genome in a biological sample,
comprising the following steps:

a) isolation of the DNA from the biological sample to be analyzed or production of a cDNA from the RNA of the biological sample,

b) detection of the nucleic acid sequences of the mycobacterium present in said biological sample,

d) analysis for the presence or the absence of a nucleic acid fragment according to claims 2 or 3.

30. An *in vitro* method for the detection and identification of *Mycobacterium tuberculosis* excepted *Mycobacterium tuberculosis* strain having the mutation CTG -> CGG at codon 463 of gene katG and/or excepted *Mycobacterium tuberculosis* strain having no or very few IS6110 sequences inserted in their genome in a biological sample, comprising the following steps:

a) bringing the biological sample to be analyzed into contact with at least one pair of primers selected among nucleic acid fragments according to claims 1 to 4, 7 and 8, and more preferably selected among the primers chosen from the group comprising SEQ ID N°13, SEQ ID N°14, SEQ ID N°15, SEQ ID N°16, SEQ ID N°17, SEQ ID N°18, the DNA contained in the sample having been, where appropriate, made accessible to the hybridization beforehand,

b) amplification of the DNA of the mycobacterium,

c) visualization of the amplification of the DNA fragments.

31. A kit for the detection and identification of *Mycobacterium tuberculosis* excepted *Mycobacterium tuberculosis* strain having the mutation CTG -> CGG at codon 463 of gene katG and/or excepted *Mycobacterium tuberculosis* strain having no or very few IS6110 sequences inserted in their genome in a biological sample, comprising the following elements:

a) at least one pair of primers selected among nucleic acid fragments according to claims 1 to 4, 7 and 8, and more preferably selected among the primers chosen from the group comprising SEQ ID N°13, SEQ ID N°14, SEQ ID N°15, SEQ ID N°16, SEQ ID N°17, SEQ ID N°18,

b) the reagents necessary to carry out a DNA amplification reaction,

c) optionally, the necessary components which make it possible to verify or compare the sequence and/or the size of the amplified fragment.

32. A method for the detection *in vitro* of antibodies directed against *Mycobacterium tuberculosis* excepted *Mycobacterium Tuberculosis* having the mutation CTG -> CGG at

codon 463 of gene KatG and/or having no or very few IS6110 sequences inserted in their genome, in a biological sample, comprising the following steps:

a) bringing the biological sample into contact with at least one product as defined in claim 20,

b) detecting the antigen-antibody complex formed.

33. Use of TbD1 deletion as a as a genetic marker for the differentiation of Mycobacterium strain of Mycobacterium Tuberculosis complex.

34. Use of mmpL6$^{551}$ polymorphism as a genetic marker for the differentiation of Mycobacterium strain of Mycobacterium Tuberculosis complex.

35. Use of the geneic marker according to claims 33 and/or 34 in association with at least one genetic markers selected among RD1, RD2, RD3, RD4, RD5, RD6, RD7, RD8, RD9, RD10, Rd11, RD13, RD14, RvD1, RvD2, RvD3, RvD4, RvD5, katG$^{463}$, gyrA$^{95}$, oxyR'$^{285}$, pncA$^{57}$ for the differentiation of Mycobacterium strain of Mycobacterium Tuberculosis complex.

36. An *in vitro* method for the detection and identification of *Mycobacteria* from the *Mycobacterium Tuberculosis* complex in a biological sample, comprising the following steps:

c) analysis for the presence or the absence of a nucleic acid fragment of the sequence TbD1 according to claims 2 or 3, and

d) analysis of at least one additional genetic marker selected among RD1, RD2, RD3, RD4, RD5, RD6, RD7, RD8, RD9, RD10, Rd11, RD13, RD14, RvD1, RvD2, RvD3, RvD4, RvD5, katG$^{463}$, gyrA$^{95}$, oxyR'$^{285}$, pncA$^{57}$.

37. The *in vitro* method of claim 36 wherein two additional markers are used, preferably RD4 and RD9.

38. The method according to claim 36 wherein the analysis is performed by a technique selected among sequence hybridization, nucleic acid amplification, antigen-antibody complex.

39. A kit for the detection and identification of *Mycobacteria* from the *Mycobacterium Tuberculosis* complex in a biological sample comprising the following elements:

e) at least one pair of primers selected among nucleic acid fragments according to claims 1 to 4, 7 and 8, and more preferably selected among the primers chosen from the group comprising SEQ ID N°13, SEQ ID N°14, SEQ ID N°15, SEQ ID N°16, SEQ ID N°17, SEQ ID N°18,

f) at least one pair of primers specific of the genetic markers selected among RD1, RD2, RD3, RD4, RD5, RD6, RD7, RD8, RD9, RD10, Rd11, RD13, RD14, RvD1, RvD2, RvD3, RvD4, RvD5, $katG^{463}$, $gyrA^{95}$, $oxyR'^{285}$, $pncA^{57}$.

g) the reagents necessary to carry out a DNA amplification reaction,

h) optionally, the necessary components which make it possible to verify or compare the sequence and/or the size of the amplified fragment.

40. A kit according to claim 39 comprising the following elements:

f) at least one pair of primers selected among nucleic acid fragments according to claims 1 to 4, 7 and 8, and more preferably selected among the primers chosen from the group comprising SEQ ID N°13, SEQ ID N°14, SEQ ID N°15, SEQ ID N°16, SEQ ID N°17, SEQ ID N°18,

g) one pair of primers specific of the genetic markers RD4,

h) one pair of primers specific of the genetic markers RD9,

i) the reagents necessary to carry out a DNA amplification reaction,

j) optionally, the necessary components which make it possible to verify or compare the sequence and/or the size of the amplified fragment.

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# PATENT APPLICATION

**TITLE:** **DELETED SEQUENCE IN *M. TUBERCULOSIS*, METHOD FOR DETECTING MYCOBACTERIA USING THESE SEQUENCES AND VACCINES**

**APPLICANTS:** **INSTITUT PASTEUR**

**ABSTRACT:**

The present invention is the identification of a nucleotide sequence which make it possible in particular to distinguish an infection resulting from *Mycobacterium tuberculosis* from an infection resulting from *Mycobacterium africanum, Mycobacterium canetti, Mycobacterium microti, Mycobacterium bovis, Mycobacterium bovis BCG.* The subject of the present invention is also a method for detecting the sequences in question by the products of expression of these sequences and the kits for carrying out these methods. Finally, the subject of the present invention is novel vaccines.

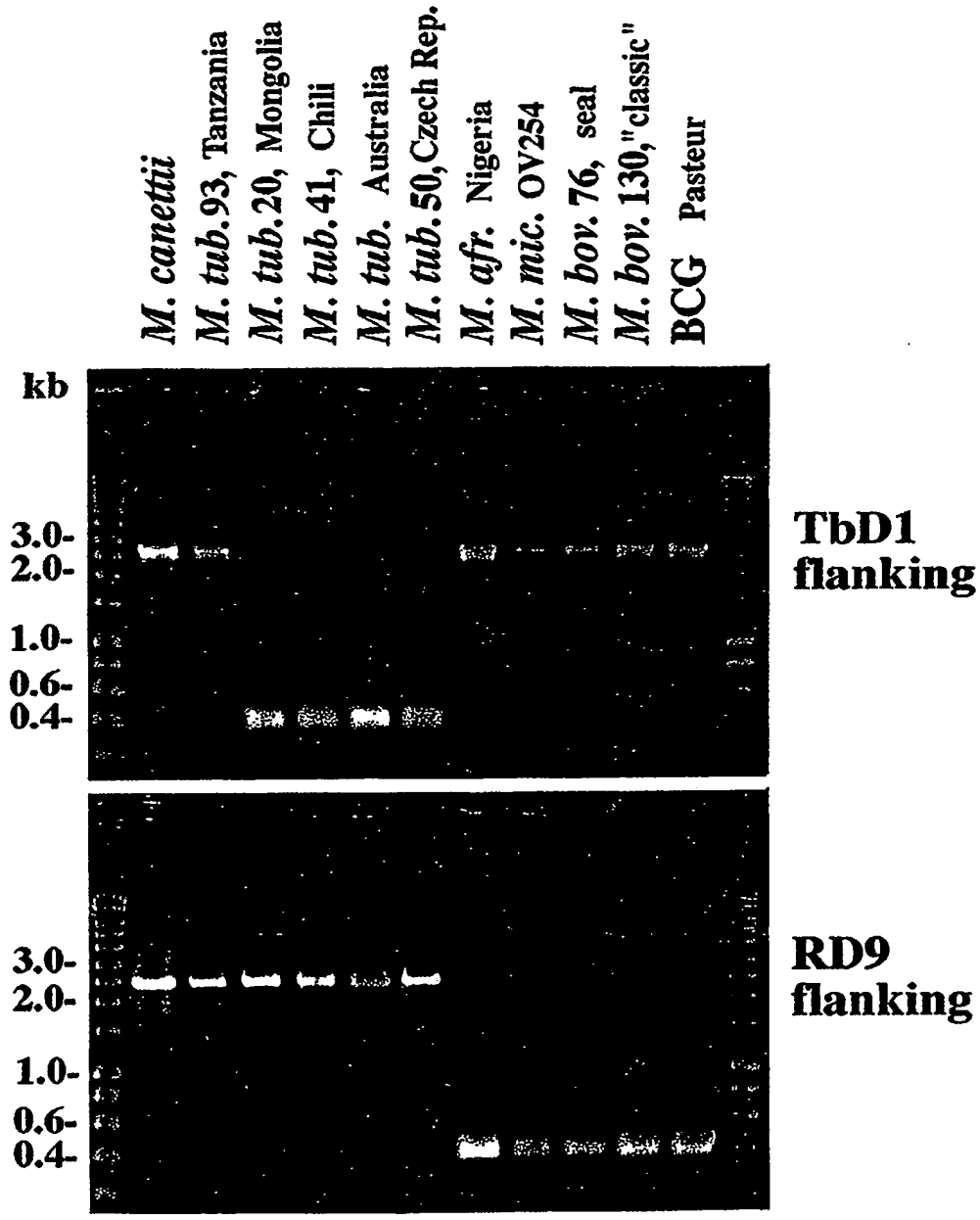
M. canettii
M. tub.93, Tanzania
M. tub.20, Mongolia
M. tub.41, Chili
M. tub. Australia
M. tub.50,Czech Rep.
M. afr. Nigeria
M. mic. OV254
M. bov.76, seal
M. bov.130,"classic"
BCG Pasteur
kb
3.0-
2.0-
1.0-
0.6-
0.4-
TbD1 flanking
3.0-
2.0-
1.0-
0.6-
0.4-
RD9 flanking

**Figure 1**

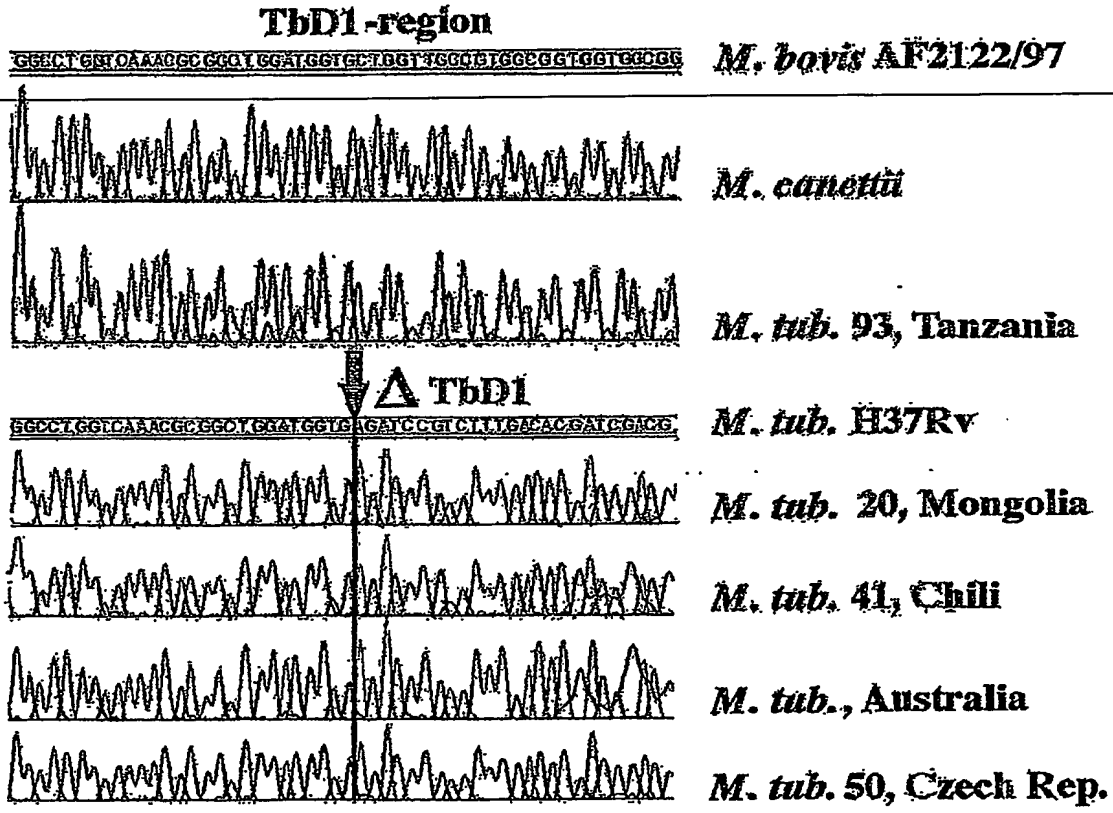
TbD1-region
M. bovis AF2122/97
M. canettii
M. tub. 93, Tanzania
Δ TbD1
M. tub. H37Rv
M. tub. 20, Mongolia
M. tub. 41, Chili
M. tub., Australia
M. tub. 50, Czech Rep.

Figure 2

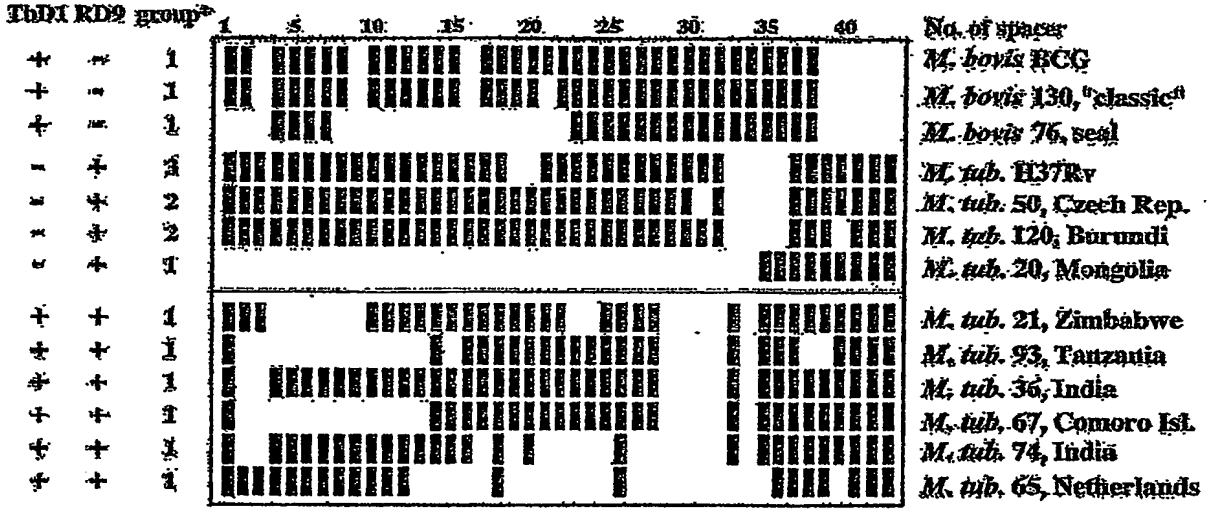


Fig. 1c: Spoligotypes and presence/absence of TbD1 and RD9 regions of selected M. tuberculosis and M. bovis strains. Numbers correspond to strain designation used in Ref. 8 and 9.

**Figure 3**

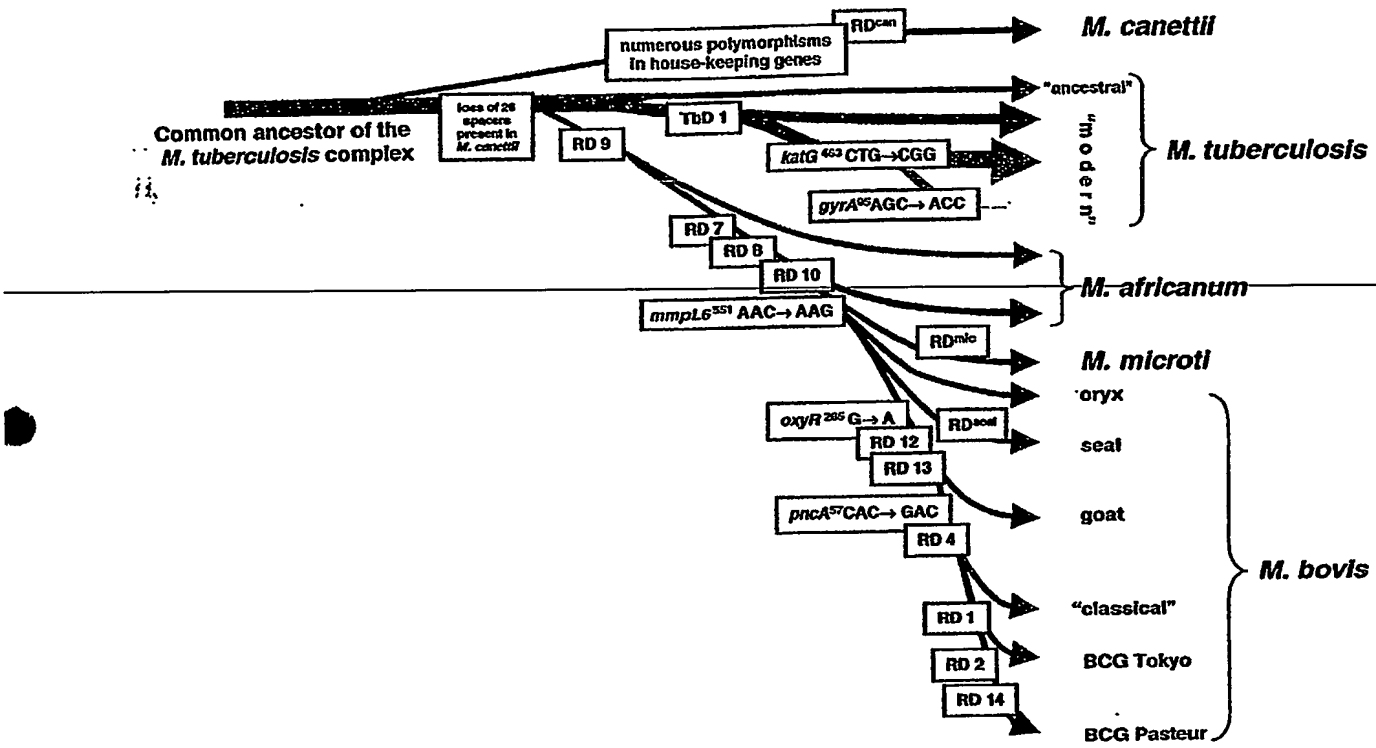
Common ancestor of the
M. tuberculosis complex
loss of 26 spacers present in M. canettii
numerous polymorphisms in house-keeping genes
RDcan
M. canettii
"ancestral"
TbD 1
RD 9
katG 463 CTG→CGG
gyrA 95 AGC→ ACC
"modern"
M. tuberculosis
RD 7
RD 8
RD 10
M. africanum
mmpL6 351 AAC→ AAG
RDmic
M. microti
oryx
oxyR 285 G→ A
RDseal
RD 12
seal
RD 13
goat
pncA 57 CAC→ GAC
RD 4
M. bovis
RD 1
"classical"
RD 2
BCG Tokyo
RD 14
BCG Pasteur

**Figure 4**

SEQUENCE LISTING

<110> Institut PASTEUR

<120> DELETED SEQUENCE IN M. TUBERCULOSIS, METHOD FOR DETECTING MYCOBACTERIA USING THESE SEQUENCES AND VACCINES

<130> D20110

<160> 20

<170> PatentIn Ver. 2.1

<210> 1
<211> 3953
<212> DNA
<213> Mycobacterium tuberculosis

<220>
<221> CDS
<222> (735)..(3638)

<400> 1
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tccatcatcc ccaaccggcc gcccacccag gacacggtgc aagccctcgt cgacgacgtg 120

atactcccca gcctcacccg atccaccggt tgagtcagcg gtgcgaatgg ctgggcaccg 180

ttgtggtgtc cggtcccgta ccgtactgtt gaatccgcgg atccccgcct gaggtacggg 240

gcgtggtcgc gccccgggca atagcgtcgc cggttatcga aaggctaacg ggtgcagggg 300

atttcagtga ctggcctggt caaacgcggc tggatggtgc tggttgccgt ggcggtggtg 360

gcggtcgcgg gattcagcgt ctatcggttg cacggcatct tcggctcgca cgacaccacc 420

tcgaccgccg gtggtgtcgc gaacgacatc aagccgttca accccaaaca ggtaaccctc 480

gaggtctttg gcgctcccgg aaccgtggca acgatcaatt atctggacgt ggatgccaca 540

cctcggcaag tcctggacac gaccctgccg tggtcataca cgatcacgac gaccctgccc 600

gcggtcttcg ccaatgttgt cgcgcaaggc gacagcaatt ccatcggctg ccgcatcacc 660

gtcaacggtg tagtcaagga cgaaaggatc gtcaacgaag tgcgcgccta taccttctgc 720

ctcgacaagt cctc atg agc aac cac cac cgc ccg cgg cct tgg ttg ccg 770
Met Ser Asn His His Arg Pro Arg Pro Trp Leu Pro
1 5 10

cac acc atc cga cgg ctt tcg ttg ccg atc ttg ctg ttt tgg gtg ggt 818
His Thr Ile Arg Arg Leu Ser Leu Pro Ile Leu Leu Phe Trp Val Gly
15 20 25

gtg gcc gcc ata acc aat gcc gcc gtg ccg caa ttg gag gtg gtc ggg 866
Val Ala Ala Ile Thr Asn Ala Ala Val Pro Gln Leu Glu Val Val Gly
30 35 40

gag gcg cat aac gtc gca cag agc tcc ccg gat gac ccg tcg ctg cag 914

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Glu Ala His Asn Val Ala Gln Ser Ser Pro Asp Asp Pro Ser Leu Gln
 45                  50                  55                  60

gcg atg aaa cgc atc ggc aag gtg ttc cac gag ttc gat tcc gac agt   962
Ala Met Lys Arg Ile Gly Lys Val Phe His Glu Phe Asp Ser Asp Ser
                 65                  70                  75

gcg gcc atg atc gtc ttg gaa ggc gat aag ccg ctc ggc aac gac gcc   1010
Ala Ala Met Ile Val Leu Glu Gly Asp Lys Pro Leu Gly Asn Asp Ala
             80                  85                  90

cac cgg ttc tac gac acc ctg ctc cgc aac ctt tca aac gac acc aaa   1058
His Arg Phe Tyr Asp Thr Leu Leu Arg Asn Leu Ser Asn Asp Thr Lys
         95                  100                 105

cac gtc gag cac gtt cag gac ttc tgg ggc gat ccg ctg acc gcg gcc   1106
His Val Glu His Val Gln Asp Phe Trp Gly Asp Pro Leu Thr Ala Ala
    110                 115                 120

ggc tcg caa agc acc gac ggc aaa gcc gcc tac gtt cag gtc tat ctc   1154
Gly Ser Gln Ser Thr Asp Gly Lys Ala Ala Tyr Val Gln Val Tyr Leu
125                 130                 135                 140

gcc ggc aac caa ggc gag gcg ttg tca atc gag tcc gtc gac gcg gtg   1202
Ala Gly Asn Gln Gly Glu Ala Leu Ser Ile Glu Ser Val Asp Ala Val
                145                 150                 155

cgc gac atc gtc gcc cat acg cca cca ccg gcc ggg gtc aag gcc tac   1250
Arg Asp Ile Val Ala His Thr Pro Pro Pro Ala Gly Val Lys Ala Tyr
            160                 165                 170

gtc acc ggc gcg gcc ccg ctc atg gcc gat cag ttt cag gtg ggc agc   1298
Val Thr Gly Ala Ala Pro Leu Met Ala Asp Gln Phe Gln Val Gly Ser
        175                 180                 185

aaa gga acc gcg aaa gtt acc ggg ata act ctg gtt gtg atc gcg gtg   1346
Lys Gly Thr Ala Lys Val Thr Gly Ile Thr Leu Val Val Ile Ala Val
    190                 195                 200

atg ttg ctc ttc gta tac cgt tcc gtc gtc acc atg gtc ctg gtg ctt   1394
Met Leu Leu Phe Val Tyr Arg Ser Val Val Thr Met Val Leu Val Leu
205                 210                 215                 220

atc acg gtt ctt att gag ttg gcc gcg gcc cgc ggg atc gtc gct ttt   1442
Ile Thr Val Leu Ile Glu Leu Ala Ala Ala Arg Gly Ile Val Ala Phe
                225                 230                 235

ctc gga aac gcc ggg gta atc ggg ctg tcg aca tac tcg acg aat ctg   1490
Leu Gly Asn Ala Gly Val Ile Gly Leu Ser Thr Tyr Ser Thr Asn Leu
            240                 245                 250

ctc aca cta ttg gta atc gcg gcg ggc aca gac tac gcg att ttt gtc   1538
Leu Thr Leu Leu Val Ile Ala Ala Gly Thr Asp Tyr Ala Ile Phe Val
        255                 260                 265

ctc ggc cgc tat cac gag gcg cgc tac gcc gca cag gat cgg gaa acg   1586
Leu Gly Arg Tyr His Glu Ala Arg Tyr Ala Ala Gln Asp Arg Glu Thr
    270                 275                 280

gcc ttc tac acg atg tat cgc ggg acc gcc cac gtc gtc ttg ggc tcg   1634
Ala Phe Tyr Thr Met Tyr Arg Gly Thr Ala His Val Val Leu Gly Ser
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285                 290                 295                 300

ggt ctg acc gtt gcc ggc gcg gtg tat tgc ctg agc ttt acc cgg cta   1682
Gly Leu Thr Val Ala Gly Ala Val Tyr Cys Leu Ser Phe Thr Arg Leu
                305                 310                 315

ccc tat ttt caa agc ctg ggt att ccc gcc tcg ata ggg gtg atg att   1730
Pro Tyr Phe Gln Ser Leu Gly Ile Pro Ala Ser Ile Gly Val Met Ile
            320                 325                 330

gcg ttg gca gcc gcg ctc agc ctg gcc cca tcc gtg ctc atc ttg ggc   1778
Ala Leu Ala Ala Ala Leu Ser Leu Ala Pro Ser Val Leu Ile Leu Gly
        335                 340                 345

agt cgt ttc ggt tgt ttc gaa ccc aag cgc agg atg agg acc agg gga   1826
Ser Arg Phe Gly Cys Phe Glu Pro Lys Arg Arg Met Arg Thr Arg Gly
    350                 355                 360

tgg cgg cgc atc ggc acg gcc atc gtg cgt tgg ccg gga ccc atc ctg   1874
Trp Arg Arg Ile Gly Thr Ala Ile Val Arg Trp Pro Gly Pro Ile Leu
365                 370                 375                 380

gca gtg gcg tgc gca att gcg gtg gtg ggt ctg ctc gcg ctg ccg gga   1922
Ala Val Ala Cys Ala Ile Ala Val Val Gly Leu Leu Ala Leu Pro Gly
                385                 390                 395

tac aaa acg agc tac gac gct cgc tat tac atg ccc gcc acc gcc ccg   1970
Tyr Lys Thr Ser Tyr Asp Ala Arg Tyr Tyr Met Pro Ala Thr Ala Pro
            400                 405                 410

gcc aat att ggc tac atg gcc gcg gag cga cat ttt ccc caa gcg cgg   2018
Ala Asn Ile Gly Tyr Met Ala Ala Glu Arg His Phe Pro Gln Ala Arg
        415                 420                 425

ctg aat ccc gaa cta ctg atg atc gag acg gat cac gat atg cgc aat   2066
Leu Asn Pro Glu Leu Leu Met Ile Glu Thr Asp His Asp Met Arg Asn
    430                 435                 440

ccg gcc gac atg ctc atc ttg gat agg atc gcc aag gct gtc ttc cat   2114
Pro Ala Asp Met Leu Ile Leu Asp Arg Ile Ala Lys Ala Val Phe His
445                 450                 455                 460

ctg ccc ggc ata ggg ctg gtg cag gcc atg acc cgg ccg cta gga acc   2162
Leu Pro Gly Ile Gly Leu Val Gln Ala Met Thr Arg Pro Leu Gly Thr
                465                 470                 475

ccg att gac cac agc tcg ata ccg ttt cag atc agc atg caa agc gtc   2210
Pro Ile Asp His Ser Ser Ile Pro Phe Gln Ile Ser Met Gln Ser Val
            480                 485                 490

ggc cag att cag aat ctc aag tat cag agg gac cga gca gcc gac ttg   2258
Gly Gln Ile Gln Asn Leu Lys Tyr Gln Arg Asp Arg Ala Ala Asp Leu
        495                 500                 505

ctg aag cag gcc gaa gag ctg ggg aag acg atc gaa atc ttg cag cgc   2306
Leu Lys Gln Ala Glu Glu Leu Gly Lys Thr Ile Glu Ile Leu Gln Arg
    510                 515                 520

caa tat gcc cta cag cag gaa ctc gcg gcc gct act cac gag caa gcc   2354
Gln Tyr Ala Leu Gln Gln Glu Leu Ala Ala Ala Thr His Glu Gln Ala
525                 530                 535                 540
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gaa agc ttt cac caa acg atc gcc acg gta aac gaa ctg cga gat agg   2402
Glu Ser Phe His Gln Thr Ile Ala Thr Val Asn Glu Leu Arg Asp Arg
                545                 550                 555

atc gcc aat ttc gac gat ttc ttc agg ccg att cgt agt tac ttt tac   2450
Ile Ala Asn Phe Asp Asp Phe Phe Arg Pro Ile Arg Ser Tyr Phe Tyr
            560                 565                 570

tgg gaa aag cac tgc tac gat atc ccg agc tgc tgg gcg ctg aga tcc   2498
Trp Glu Lys His Cys Tyr Asp Ile Pro Ser Cys Trp Ala Leu Arg Ser
        575                 580                 585

gtc ttt gac acg atc gac ggt atc gac caa ctc ggc gag cag ctg gcc   2546
Val Phe Asp Thr Ile Asp Gly Ile Asp Gln Leu Gly Glu Gln Leu Ala
    590                 595                 600

agc gtg acc gta acc ttg gac aag ttg gct gcg atc cag cct caa ttg   2594
Ser Val Thr Val Thr Leu Asp Lys Leu Ala Ala Ile Gln Pro Gln Leu
605                 610                 615                 620

gtg gcg ctg cta cca gac gag atc gcc agc cag cag atc aat cgg gaa   2642
Val Ala Leu Leu Pro Asp Glu Ile Ala Ser Gln Gln Ile Asn Arg Glu
                625                 630                 635

ctg gcg ctg gct aac tac gcc acc atg tcc ggg atc tat gcc cag acg   2690
Leu Ala Leu Ala Asn Tyr Ala Thr Met Ser Gly Ile Tyr Ala Gln Thr
            640                 645                 650

gcg gcc ttg atc gaa aac gct gcc gcc atg gga caa gcc ttt gac gcc   2738
Ala Ala Leu Ile Glu Asn Ala Ala Ala Met Gly Gln Ala Phe Asp Ala
        655                 660                 665

gcc aag aac gac gac tcc ttc tat ctg ccg ccg gag gct ttt gac aac   2786
Ala Lys Asn Asp Asp Ser Phe Tyr Leu Pro Pro Glu Ala Phe Asp Asn
    670                 675                 680

cca gat ttc cag cgc ggc ctg aaa ttg ttc ctg tcg gca gac ggt aag   2834
Pro Asp Phe Gln Arg Gly Leu Lys Leu Phe Leu Ser Ala Asp Gly Lys
685                 690                 695                 700

gcg gct cgg atg atc atc tcc cat gaa ggc gat ccc gcc acc ccc gaa   2882
Ala Ala Arg Met Ile Ile Ser His Glu Gly Asp Pro Ala Thr Pro Glu
                705                 710                 715

ggc att tcg cat atc gac gcg atc aag cag gcg gcc cac gag gcc gtg   2930
Gly Ile Ser His Ile Asp Ala Ile Lys Gln Ala Ala His Glu Ala Val
            720                 725                 730

aag ggc act ccc atg gcg ggt gct ggg atc tat ctg gcc ggc acg gcc   2978
Lys Gly Thr Pro Met Ala Gly Ala Gly Ile Tyr Leu Ala Gly Thr Ala
        735                 740                 745

gcc acc ttc aag gac att caa gac ggc gcc acc tac gac ctc ctg atc   3026
Ala Thr Phe Lys Asp Ile Gln Asp Gly Ala Thr Tyr Asp Leu Leu Ile
    750                 755                 760

gcc gga ata gcc gcg ctg agc ttg att ttg ctc atc atg atg atc att   3074
Ala Gly Ile Ala Ala Leu Ser Leu Ile Leu Leu Ile Met Met Ile Ile
765                 770                 775                 780
```

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acc cga agc ctg gtt gcg gcg ctg gtg atc gtg ggc acg gtg gcg ctg   3122
Thr Arg Ser Leu Val Ala Ala Leu Val Ile Val Gly Thr Val Ala Leu
                785                 790                 795

tcg ttg ggc gct tct ttt ggc ctg tcc gtg ctg gtg tgg cag cat ctt   3170
Ser Leu Gly Ala Ser Phe Gly Leu Ser Val Leu Val Trp Gln His Leu
            800                 805                 810

ctc ggt atc cag ttg tac tgg atc gtg ctc gcg ctg gcc gtc atc ctg   3218
Leu Gly Ile Gln Leu Tyr Trp Ile Val Leu Ala Leu Ala Val Ile Leu
        815                 820                 825

ctc ctg gcc gtg gga tcg gac tat aac ttg ctg ctg att tcc cga ttc   3266
Leu Leu Ala Val Gly Ser Asp Tyr Asn Leu Leu Leu Ile Ser Arg Phe
    830                 835                 840

aag gag gag atc ggt gca ggt ttg aac acc ggc atc atc cgt gcg atg   3314
Lys Glu Glu Ile Gly Ala Gly Leu Asn Thr Gly Ile Ile Arg Ala Met
845                 850                 855                 860

gcc ggc acc ggc ggg gtg gtg acc gct gcc ggc ctg gtg ttc gcc gcc   3362
Ala Gly Thr Gly Gly Val Val Thr Ala Ala Gly Leu Val Phe Ala Ala
                865                 870                 875

act atg tct tcg ttc gtg ttc agt gat ttg cgg gtc ctc ggt cag atc   3410
Thr Met Ser Ser Phe Val Phe Ser Asp Leu Arg Val Leu Gly Gln Ile
            880                 885                 890

ggg acc acc att ggt ctt ggg ctg ctg ttc gac acg ctg gtg gtg cgc   3458
Gly Thr Thr Ile Gly Leu Gly Leu Leu Phe Asp Thr Leu Val Val Arg
        895                 900                 905

gcg ttc atg acc ccg tcc atc gcg gtg ctg ctc ggg cgc tgg ttc tgg   3506
Ala Phe Met Thr Pro Ser Ile Ala Val Leu Leu Gly Arg Trp Phe Trp
    910                 915                 920

tgg ccg caa cga gtg cgc ccg cgc cct gcc agc agg atg ctt cgg ccg   3554
Trp Pro Gln Arg Val Arg Pro Arg Pro Ala Ser Arg Met Leu Arg Pro
925                 930                 935                 940

tac ggc ccg cgg ccc gtg gtt cgt gaa ttg ctg ctg cgc gag ggc aac   3602
Tyr Gly Pro Arg Pro Val Val Arg Glu Leu Leu Leu Arg Glu Gly Asn
                945                 950                 955

gat gac ccg aga act cag gtg gct acc cac cgt taa ggtggtggga        3648
Asp Asp Pro Arg Thr Gln Val Ala Thr His Arg
            960                 965

tgccgctttc aggggaatat gcgccgagcc cgctcgactg gtcgcgcgag caagccgaca 3708

cgtatatgaa gtccggcgga accgagggca cacagctgca gggaaagccg gtcatcctgc 3768

tcaccaccgt cggggcgaag accggcaaac tccgtaagac cccgctgatg cgcgtcgagc 3828

acgacggcca gtacgcgatc gtcgcctcgc tgggtggggc gccgaaaaat ccggtctggt 3888

accacaacgt cgtgaagaac ccacgggtcg agctgcagga cggcaccgga ccggcgacta 3948

cgacg                                                             3953
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<210> 2
<211> 967
<212> PRT
<213> Mycobacterium tuberculosis

<220>
<223> mmpL6 protein

<400> 2
Met Ser Asn His His Arg Pro Arg Pro Trp Leu Pro His Thr Ile Arg
1 5 10 15

Arg Leu Ser Leu Pro Ile Leu Leu Phe Trp Val Gly Val Ala Ala Ile
20 25 30

Thr Asn Ala Ala Val Pro Gln Leu Glu Val Val Gly Glu Ala His Asn
35 40 45

Val Ala Gln Ser Ser Pro Asp Asp Pro Ser Leu Gln Ala Met Lys Arg
50 55 60

Ile Gly Lys Val Phe His Glu Phe Asp Ser Asp Ser Ala Ala Met Ile
65 70 75 80

Val Leu Glu Gly Asp Lys Pro Leu Gly Asn Asp Ala His Arg Phe Tyr
85 90 95

Asp Thr Leu Leu Arg Asn Leu Ser Asn Asp Thr Lys His Val Glu His
100 105 110

Val Gln Asp Phe Trp Gly Asp Pro Leu Thr Ala Ala Gly Ser Gln Ser
115 120 125

Thr Asp Gly Lys Ala Ala Tyr Val Gln Val Tyr Leu Ala Gly Asn Gln
130 135 140

Gly Glu Ala Leu Ser Ile Glu Ser Val Asp Ala Val Arg Asp Ile Val
145 150 155 160

Ala His Thr Pro Pro Pro Ala Gly Val Lys Ala Tyr Val Thr Gly Ala
165 170 175

Ala Pro Leu Met Ala Asp Gln Phe Gln Val Gly Ser Lys Gly Thr Ala
180 185 190

Lys Val Thr Gly Ile Thr Leu Val Val Ile Ala Val Met Leu Leu Phe
195 200 205

Val Tyr Arg Ser Val Val Thr Met Val Leu Val Leu Ile Thr Val Leu
210 215 220

Ile Glu Leu Ala Ala Ala Arg Gly Ile Val Ala Phe Leu Gly Asn Ala
225 230 235 240

Gly Val Ile Gly Leu Ser Thr Tyr Ser Thr Asn Leu Leu Thr Leu Leu
245 250 255

Val Ile Ala Ala Gly Thr Asp Tyr Ala Ile Phe Val Leu Gly Arg Tyr
260 265 270

His Glu Ala Arg Tyr Ala Ala Gln Asp Arg Glu Thr Ala Phe Tyr Thr

275 280 285

Met Tyr Arg Gly Thr Ala His Val Val Leu Gly Ser Gly Leu Thr Val
290 295 300

Ala Gly Ala Val Tyr Cys Leu Ser Phe Thr Arg Leu Pro Tyr Phe Gln
305 310 315 320

Ser Leu Gly Ile Pro Ala Ser Ile Gly Val Met Ile Ala Leu Ala Ala
325 330 335

Ala Leu Ser Leu Ala Pro Ser Val Leu Ile Leu Gly Ser Arg Phe Gly
340 345 350

Cys Phe Glu Pro Lys Arg Arg Met Arg Thr Arg Gly Trp Arg Arg Ile
355 360 365

Gly Thr Ala Ile Val Arg Trp Pro Gly Pro Ile Leu Ala Val Ala Cys
370 375 380

Ala Ile Ala Val Val Gly Leu Leu Ala Leu Pro Gly Tyr Lys Thr Ser
385 390 395 400

Tyr Asp Ala Arg Tyr Tyr Met Pro Ala Thr Ala Pro Ala Asn Ile Gly
405 410 415

Tyr Met Ala Ala Glu Arg His Phe Pro Gln Ala Arg Leu Asn Pro Glu
420 425 430

Leu Leu Met Ile Glu Thr Asp His Asp Met Arg Asn Pro Ala Asp Met
435 440 445

Leu Ile Leu Asp Arg Ile Ala Lys Ala Val Phe His Leu Pro Gly Ile
450 455 460

Gly Leu Val Gln Ala Met Thr Arg Pro Leu Gly Thr Pro Ile Asp His
465 470 475 480

Ser Ser Ile Pro Phe Gln Ile Ser Met Gln Ser Val Gly Gln Ile Gln
485 490 495

Asn Leu Lys Tyr Gln Arg Asp Arg Ala Ala Asp Leu Leu Lys Gln Ala
500 505 510

Glu Glu Leu Gly Lys Thr Ile Glu Ile Leu Gln Arg Gln Tyr Ala Leu
515 520 525

Gln Gln Glu Leu Ala Ala Ala Thr His Glu Gln Ala Glu Ser Phe His
530 535 540

Gln Thr Ile Ala Thr Val Asn Glu Leu Arg Asp Arg Ile Ala Asn Phe
545 550 555 560

Asp Asp Phe Phe Arg Pro Ile Arg Ser Tyr Phe Tyr Trp Glu Lys His
565 570 575

Cys Tyr Asp Ile Pro Ser Cys Trp Ala Leu Arg Ser Val Phe Asp Thr
580 585 590

Ile Asp Gly Ile Asp Gln Leu Gly Glu Gln Leu Ala Ser Val Thr Val
595 600 605

```
Thr Leu Asp Lys Leu Ala Ala Ile Gln Pro Gln Leu Val Ala Leu Leu
    610                 615                 620

Pro Asp Glu Ile Ala Ser Gln Gln Ile Asn Arg Glu Leu Ala Leu Ala
625                 630                 635                 640

Asn Tyr Ala Thr Met Ser Gly Ile Tyr Ala Gln Thr Ala Ala Leu Ile
                645                 650                 655

Glu Asn Ala Ala Ala Met Gly Gln Ala Phe Asp Ala Ala Lys Asn Asp
            660                 665                 670

Asp Ser Phe Tyr Leu Pro Pro Glu Ala Phe Asp Asn Pro Asp Phe Gln
        675                 680                 685

Arg Gly Leu Lys Leu Phe Leu Ser Ala Asp Gly Lys Ala Ala Arg Met
    690                 695                 700

Ile Ile Ser His Glu Gly Asp Pro Ala Thr Pro Glu Gly Ile Ser His
705                 710                 715                 720

Ile Asp Ala Ile Lys Gln Ala Ala His Glu Ala Val Lys Gly Thr Pro
                725                 730                 735

Met Ala Gly Ala Gly Ile Tyr Leu Ala Gly Thr Ala Ala Thr Phe Lys
            740                 745                 750

Asp Ile Gln Asp Gly Ala Thr Tyr Asp Leu Leu Ile Ala Gly Ile Ala
        755                 760                 765

Ala Leu Ser Leu Ile Leu Leu Ile Met Met Ile Ile Thr Arg Ser Leu
    770                 775                 780

Val Ala Ala Leu Val Ile Val Gly Thr Val Ala Leu Ser Leu Gly Ala
785                 790                 795                 800

Ser Phe Gly Leu Ser Val Leu Val Trp Gln His Leu Leu Gly Ile Gln
                805                 810                 815

Leu Tyr Trp Ile Val Leu Ala Leu Ala Val Ile Leu Leu Leu Ala Val
            820                 825                 830

Gly Ser Asp Tyr Asn Leu Leu Leu Ile Ser Arg Phe Lys Glu Glu Ile
        835                 840                 845

Gly Ala Gly Leu Asn Thr Gly Ile Ile Arg Ala Met Ala Gly Thr Gly
    850                 855                 860

Gly Val Val Thr Ala Ala Gly Leu Val Phe Ala Ala Thr Met Ser Ser
865                 870                 875                 880

Phe Val Phe Ser Asp Leu Arg Val Leu Gly Gln Ile Gly Thr Thr Ile
                885                 890                 895

Gly Leu Gly Leu Leu Phe Asp Thr Leu Val Val Arg Ala Phe Met Thr
            900                 905                 910

Pro Ser Ile Ala Val Leu Leu Gly Arg Trp Phe Trp Trp Pro Gln Arg
        915                 920                 925
```

```
Val Arg Pro Arg Pro Ala Ser Arg Met Leu Arg Pro Tyr Gly Pro Arg
    930                 935                 940

Pro Val Val Arg Glu Leu Leu Leu Arg Glu Gly Asn Asp Asp Pro Arg
945                 950                 955                 960

Thr Gln Val Ala Thr His Arg
                965
```

<210> 3
<211> 148
<212> PRT
<213> Mycobacterium tuberculosis

<220>
<223> mmpS6 protein

<400> 3

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Val Gln Gly Ile Ser Val Thr Gly Leu Val Lys Arg Gly Trp Met Val
  1               5                  10                  15

Leu Val Ala Val Ala Val Val Ala Val Ala Gly Phe Ser Val Tyr Arg
             20                  25                  30

Leu His Gly Ile Phe Gly Ser His Asp Thr Thr Ser Thr Ala Gly Gly
         35                  40                  45

Val Ala Asn Asp Ile Lys Pro Phe Asn Pro Lys Gln Val Thr Leu Glu
     50                  55                  60

Val Phe Gly Ala Pro Gly Thr Val Ala Thr Ile Asn Tyr Leu Asp Val
 65                  70                  75                  80

Asp Ala Thr Pro Arg Gln Val Leu Asp Thr Thr Leu Pro Trp Ser Tyr
                 85                  90                  95

Thr Ile Thr Thr Thr Leu Pro Ala Val Phe Ala Asn Val Val Ala Gln
            100                 105                 110

Gly Asp Ser Asn Ser Ile Gly Cys Arg Ile Thr Val Asn Gly Val Val
        115                 120                 125

Lys Asp Glu Arg Ile Val Asn Glu Val Arg Ala Tyr Thr Phe Cys Leu
    130                 135                 140

Asp Lys Ser Ser
145
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<210> 4
<211> 2153
<212> DNA
<213> Mycobacterium tuberculosis

<400> 4

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ctggttgccg tggcggtggt ggcggtcgcg ggattcagcg tctatcggtt gcacggcatc 60
ttcggctcgc acgacaccac ctcgaccgcc ggtggtgtcg cgaacgacat caagccgttc 120
aaccccaaac aggtaaccct cgaggtcttt ggcgctcccg gaaccgtggc aacgatcaat 180
tatctggacg tggatgccac acctcggcaa gtcctggaca cgaccctgcc gtggtcatac 240
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acgatcacga cgaccctgcc cgcggtcttc gccaatgttg tcgcgcaagg cgacagcaat 300
tccatcggct gccgcatcac cgtcaacggt gtagtcaagg acgaaaggat cgtcaacgaa 360
gtgcgcgcct ataccttctg cctcgacaag tcctcatgag caaccaccac cgcccgcggc 420
cttggttgcc gcacaccatc cgacggcttt cgttgccgat cttgctgttt tgggtgggtg 480
tggccgccat aaccaatgcc gccgtgccgc aattggaggt ggtcggggag gcgcataacg 540
tcgcacagag ctccccggat gacccgtcgc tgcaggcgat gaaacgcatc ggcaaggtgt 600
tccacgagtt cgattccgac agtgcggcca tgatcgtctt ggaaggcgat aagccgctcg 660
gcaacgacgc ccaccggttc tacgacaccc tgctccgcaa cctttcaaac gacaccaaac 720
acgtcgagca cgttcaggac ttctggggcg atccgctgac cgcggccggc tcgcaaagca 780
ccgacggcaa agccgcctac gttcaggtct atctcgccgg caaccaaggc gaggcgttgt 840
caatcgagtc cgtcgacgcg gtgcgcgaca tcgtcgccca tacgccacca ccggccgggg 900
tcaaggccta cgtcaccggc gcggccccgc tcatggccga tcagtttcag gtgggcagca 960
aaggaaccgc gaaagttacc gggataactc tggttgtgat cgcggtgatg ttgctcttcg 1020
tataccgttc cgtcgtcacc atggtcctgg tgcttatcac ggttcttatt gagttggccg 1080
cggcccgcgg gatcgtcgct tttctcggaa acgccggggt aatcgggctg tcgacatact 1140
cgacgaatct gctcacacta ttggtaatcg cggcgggcac agactacgcg atttttgtcc 1200
tcggccgcta tcacgaggcg cgctacgccg cacaggatcg ggaaacggcc ttctacacga 1260
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ccaccgcccc ggccaatatt ggctacatgg ccgcggagcg acattttccc caagcgcggc 1680
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tcatcttgga taggatcgcc aaggctgtct tccatctgcc cggcataggg ctggtgcagg 1800
ccatgacccg gccgctagga accccgattg accacagctc gataccgttt cagatcagca 1860
tgcaaagcgt cggccagatt cagaatctca agtatcagag ggaccgagca gccgacttgc 1920
tgaagcaggc cgaagagctg gggaagacga tcgaaatctt gcagcgccaa tatgccctac 1980
agcaggaact cgcggccgct actcacgagc aagccgaaag ctttcaccaa acgatcgcca 2040
cggtaaacga actgcgagat aggatcgcca atttcgacga tttcttcagg ccgattcgta 2100
gttactttta ctgggaaaag cactgctacg atatcccgag ctgctgggcg ctg         2153
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<210> 5
<211> 2904
<212> DNA
<213> Mycobacterium tuberculosis

<220>
<223> mmpL6 sequence and protein

<220>
<221> CDS
<222> (1)..(2901)

<400> 5

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atg agc aac cac cac cgc ccg cgg cct tgg ttg ccg cac acc atc cga   48
Met Ser Asn His His Arg Pro Arg Pro Trp Leu Pro His Thr Ile Arg
  1               5                  10                  15

cgg ctt tcg ttg ccg atc ttg ctg ttt tgg gtg ggt gtg gcc gcc ata   96
Arg Leu Ser Leu Pro Ile Leu Leu Phe Trp Val Gly Val Ala Ala Ile
             20                  25                  30

acc aat gcc gcc gtg ccg caa ttg gag gtg gtc ggg gag gcg cat aac   144
Thr Asn Ala Ala Val Pro Gln Leu Glu Val Val Gly Glu Ala His Asn
         35                  40                  45

gtc gca cag agc tcc ccg gat gac ccg tcg ctg cag gcg atg aaa cgc   192
Val Ala Gln Ser Ser Pro Asp Asp Pro Ser Leu Gln Ala Met Lys Arg
```

```
     50                  55                  60

atc ggc aag gtg ttc cac gag ttc gat tcc gac agt gcg gcc atg atc   240
Ile Gly Lys Val Phe His Glu Phe Asp Ser Asp Ser Ala Ala Met Ile
 65                  70                  75                  80

gtc ttg gaa ggc gat aag ccg ctc ggc aac gac gcc cac cgg ttc tac   288
Val Leu Glu Gly Asp Lys Pro Leu Gly Asn Asp Ala His Arg Phe Tyr
                 85                  90                  95

gac acc ctg ctc cgc aac ctt tca aac gac acc aaa cac gtc gag cac   336
Asp Thr Leu Leu Arg Asn Leu Ser Asn Asp Thr Lys His Val Glu His
            100                 105                 110

gtt cag gac ttc tgg ggc gat ccg ctg acc gcg gcc ggc tcg caa agc   384

Val Gln Asp Phe Trp Gly Asp Pro Leu Thr Ala Ala Gly Ser Gln Ser
        115                 120                 125

acc gac ggc aaa gcc gcc tac gtt cag gtc tat ctc gcc ggc aac caa   432
Thr Asp Gly Lys Ala Ala Tyr Val Gln Val Tyr Leu Ala Gly Asn Gln
    130                 135                 140

ggc gag gcg ttg tca atc gag tcc gtc gac gcg gtg cgc gac atc gtc   480
Gly Glu Ala Leu Ser Ile Glu Ser Val Asp Ala Val Arg Asp Ile Val
145                 150                 155                 160

gcc cat acg cca cca ccg gcc ggg gtc aag gcc tac gtc acc ggc gcg   528
Ala His Thr Pro Pro Pro Ala Gly Val Lys Ala Tyr Val Thr Gly Ala
                165                 170                 175

gcc ccg ctc atg gcc gat cag ttt cag gtg ggc agc aaa gga acc gcg   576
Ala Pro Leu Met Ala Asp Gln Phe Gln Val Gly Ser Lys Gly Thr Ala
            180                 185                 190

aaa gtt acc ggg ata act ctg gtt gtg atc gcg gtg atg ttg ctc ttc   624
Lys Val Thr Gly Ile Thr Leu Val Val Ile Ala Val Met Leu Leu Phe
        195                 200                 205

gta tac cgt tcc gtc gtc acc atg gtc ctg gtg ctt atc acg gtt ctt   672
Val Tyr Arg Ser Val Val Thr Met Val Leu Val Leu Ile Thr Val Leu
    210                 215                 220

att gag ttg gcc gcg gcc cgc ggg atc gtc gct ttt ctc gga aac gcc   720
Ile Glu Leu Ala Ala Ala Arg Gly Ile Val Ala Phe Leu Gly Asn Ala
225                 230                 235                 240

ggg gta atc ggg ctg tcg aca tac tcg acg aat ctg ctc aca cta ttg   768
Gly Val Ile Gly Leu Ser Thr Tyr Ser Thr Asn Leu Leu Thr Leu Leu
                245                 250                 255   -

gta atc gcg gcg ggc aca gac tac gcg att ttt gtc ctc ggc cgc tat   816
Val Ile Ala Ala Gly Thr Asp Tyr Ala Ile Phe Val Leu Gly Arg Tyr
            260                 265                 270

cac gag gcg cgc tac gcc gca cag gat cgg gaa acg gcc ttc tac acg   864
His Glu Ala Arg Tyr Ala Ala Gln Asp Arg Glu Thr Ala Phe Tyr Thr
        275                 280                 285

atg tat cgc ggg acc gcc cac gtc gtc ttg ggc tcg ggt ctg acc gtt   912
Met Tyr Arg Gly Thr Ala His Val Val Leu Gly Ser Gly Leu Thr Val
```

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    290                 295                 300

gcc ggc gcg gtg tat tgc ctg agc ttt acc cgg cta ccc tat ttt caa   960
Ala Gly Ala Val Tyr Cys Leu Ser Phe Thr Arg Leu Pro Tyr Phe Gln
305                 310                 315                 320

agc ctg ggt att ccc gcc tcg ata ggg gtg atg att gcg ttg gca gcc   1008
Ser Leu Gly Ile Pro Ala Ser Ile Gly Val Met Ile Ala Leu Ala Ala
                325                 330                 335

gcg ctc agc ctg gcc cca tcc gtg ctc atc ttg ggc agt cgt ttc ggt   1056
Ala Leu Ser Leu Ala Pro Ser Val Leu Ile Leu Gly Ser Arg Phe Gly
            340                 345                 350

tgt ttc gaa ccc aag cgc agg atg agg acc agg gga tgg cgg cgc atc   1104
Cys Phe Glu Pro Lys Arg Arg Met Arg Thr Arg Gly Trp Arg Arg Ile
        355                 360                 365

ggc acg gcc atc gtg cgt tgg ccg gga ccc atc ctg gca gtg gcg tgc   1152
Gly Thr Ala Ile Val Arg Trp Pro Gly Pro Ile Leu Ala Val Ala Cys
    370                 375                 380

gca att gcg gtg gtg ggt ctg ctc gcg ctg ccg gga tac aaa acg agc   1200
Ala Ile Ala Val Val Gly Leu Leu Ala Leu Pro Gly Tyr Lys Thr Ser
385                 390                 395                 400

tac gac gct cgc tat tac atg ccc gcc acc gcc ccg gcc aat att ggc   1248
Tyr Asp Ala Arg Tyr Tyr Met Pro Ala Thr Ala Pro Ala Asn Ile Gly
                405                 410                 415

tac atg gcc gcg gag cga cat ttt ccc caa gcg cgg ctg aat ccc gaa   1296
Tyr Met Ala Ala Glu Arg His Phe Pro Gln Ala Arg Leu Asn Pro Glu
            420                 425                 430

cta ctg atg atc gag acg gat cac gat atg cgc aat ccg gcc gac atg   1344
Leu Leu Met Ile Glu Thr Asp His Asp Met Arg Asn Pro Ala Asp Met
        435                 440                 445

ctc atc ttg gat agg atc gcc aag gct gtc ttc cat ctg ccc ggc ata   1392
Leu Ile Leu Asp Arg Ile Ala Lys Ala Val Phe His Leu Pro Gly Ile
    450                 455                 460

ggg ctg gtg cag gcc atg acc cgg ccg cta gga acc ccg att gac cac   1440
Gly Leu Val Gln Ala Met Thr Arg Pro Leu Gly Thr Pro Ile Asp His
465                 470                 475                 480

agc tcg ata ccg ttt cag atc agc atg caa agc gtc ggc cag att cag   1488
Ser Ser Ile Pro Phe Gln Ile Ser Met Gln Ser Val Gly Gln Ile Gln
                485                 490                 495

aat ctc aag tat cag agg gac cga gca gcc gac ttg ctg aag cag gcc   1536
Asn Leu Lys Tyr Gln Arg Asp Arg Ala Ala Asp Leu Leu Lys Gln Ala
            500                 505                 510

gaa gag ctg ggg aag acg atc gaa atc ttg cag cgc caa tat gcc cta   1584
Glu Glu Leu Gly Lys Thr Ile Glu Ile Leu Gln Arg Gln Tyr Ala Leu
        515                 520                 525

cag cag gaa ctc gcg gcc gct act cac gag caa gcc gaa agc ttt cac   1632
Gln Gln Glu Leu Ala Ala Ala Thr His Glu Gln Ala Glu Ser Phe His
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    530                 535                 540

caa acg atc gcc acg gta aag gaa ctg cga gat agg atc gcc aat ttc   1680
Gln Thr Ile Ala Thr Val Lys Glu Leu Arg Asp Arg Ile Ala Asn Phe
545                 550                 555                 560

gac gat ttc ttc agg ccg att cgt agt tac ttt tac tgg gaa aag cac   1728
Asp Asp Phe Phe Arg Pro Ile Arg Ser Tyr Phe Tyr Trp Glu Lys His
                565                 570                 575

tgc tac gat atc ccg agc tgc tgg gcg ctg aga tcc gtc ttt gac acg   1776
Cys Tyr Asp Ile Pro Ser Cys Trp Ala Leu Arg Ser Val Phe Asp Thr
            580                 585                 590

atc gac ggt atc gac caa ctc ggc gag cag ctg gcc agc gtg acc gta   1824
Ile Asp Gly Ile Asp Gln Leu Gly Glu Gln Leu Ala Ser Val Thr Val
        595                 600                 605

acc ttg gac aag ttg gct gcg atc cag cct caa ttg gtg gcg ctg cta   1872
Thr Leu Asp Lys Leu Ala Ala Ile Gln Pro Gln Leu Val Ala Leu Leu
    610                 615                 620

cca gac gag atc gcc agc cag cag atc aat cgg gaa ctg gcg ctg gct   1920
Pro Asp Glu Ile Ala Ser Gln Gln Ile Asn Arg Glu Leu Ala Leu Ala
625                 630                 635                 640

aac tac gcc acc atg tcc ggg atc tat gcc cag acg gcg gcc ttg atc   1968
Asn Tyr Ala Thr Met Ser Gly Ile Tyr Ala Gln Thr Ala Ala Leu Ile
                645                 650                 655

gaa aac gct gcc gcc atg gga caa gcc ttt gac gcc gcc aag aac gac   2016
Glu Asn Ala Ala Ala Met Gly Gln Ala Phe Asp Ala Ala Lys Asn Asp
            660                 665                 670

gac tcc ttc tat ctg ccg ccg gag gct ttt gac aac cca gat ttc cag   2064
Asp Ser Phe Tyr Leu Pro Pro Glu Ala Phe Asp Asn Pro Asp Phe Gln
        675                 680                 685

cgc ggc ctg aaa ttg ttc ctg tcg gca gac ggt aag gcg gct cgg atg   2112
Arg Gly Leu Lys Leu Phe Leu Ser Ala Asp Gly Lys Ala Ala Arg Met
    690                 695                 700

atc atc tcc cat gaa ggc gat ccc gcc acc ccc gaa ggc att tcg cat   2160
Ile Ile Ser His Glu Gly Asp Pro Ala Thr Pro Glu Gly Ile Ser His
705                 710                 715                 720

atc gac gcg atc aag cag gcg gcc cac gag gcc gtg aag ggc act ccc   2208
Ile Asp Ala Ile Lys Gln Ala Ala His Glu Ala Val Lys Gly Thr Pro
                725                 730                 735

atg gcg ggt gct ggg atc tat ctg gcc ggc acg gcc gcc acc ttc aag   2256
Met Ala Gly Ala Gly Ile Tyr Leu Ala Gly Thr Ala Ala Thr Phe Lys
            740                 745                 750

gac att caa gac ggc gcc acc tac gac ctc ctg atc gcc gga ata gcc   2304
Asp Ile Gln Asp Gly Ala Thr Tyr Asp Leu Leu Ile Ala Gly Ile Ala
        755                 760                 765

gcg ctg agc ttg att ttg ctc atc atg atg atc att acc cga agc ctg   2352
Ala Leu Ser Leu Ile Leu Leu Ile Met Met Ile Ile Thr Arg Ser Leu
    770                 775                 780
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gtt gcg gcg ctg gtg atc gtg ggc acg gtg gcg ctg tcg ttg ggc gct   2400
Val Ala Ala Leu Val Ile Val Gly Thr Val Ala Leu Ser Leu Gly Ala
785                 790                 795                 800

tct ttt ggc ctg tcc gtg ctg gtg tgg cag cat ctt ctc ggt atc cag   2448
Ser Phe Gly Leu Ser Val Leu Val Trp Gln His Leu Leu Gly Ile Gln
                805                 810                 815

ttg tac tgg atc gtg ctc gcg ctg gcc gtc atc ctg ctc ctg gcc gtg   2496
Leu Tyr Trp Ile Val Leu Ala Leu Ala Val Ile Leu Leu Leu Ala Val
            820                 825                 830

gga tcg gac tat aac ttg ctg ctg att tcc cga ttc aag gag gag atc   2544
Gly Ser Asp Tyr Asn Leu Leu Leu Ile Ser Arg Phe Lys Glu Glu Ile
        835                 840                 845
```

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ggt gca ggt ttg aac acc ggc atc atc cgt gcg atg gcc ggc acc ggc   2592
Gly Ala Gly Leu Asn Thr Gly Ile Ile Arg Ala Met Ala Gly Thr Gly
    850                 855                 860

ggg gtg gtg acc gct gcc ggc ctg gtg ttc gcc gcc act atg tct tcg   2640
Gly Val Val Thr Ala Ala Gly Leu Val Phe Ala Ala Thr Met Ser Ser
865                 870                 875                 880

ttc gtg ttc agt gat ttg cgg gtc ctc ggt cag atc ggg acc acc att   2688
Phe Val Phe Ser Asp Leu Arg Val Leu Gly Gln Ile Gly Thr Thr Ile
                885                 890                 895

ggt ctt ggg ctg ctg ttc gac acg ctg gtg gtg cgc gcg ttc atg acc   2736
Gly Leu Gly Leu Leu Phe Asp Thr Leu Val Val Arg Ala Phe Met Thr
            900                 905                 910

ccg tcc atc gcg gtg ctg ctc ggg cgc tgg ttc tgg tgg ccg caa cga   2784
Pro Ser Ile Ala Val Leu Leu Gly Arg Trp Phe Trp Trp Pro Gln Arg
        915                 920                 925

gtg cgc ccg cgc cct gcc agc agg atg ctt cgg ccg tac ggc ccg cgg   2832
Val Arg Pro Arg Pro Ala Ser Arg Met Leu Arg Pro Tyr Gly Pro Arg
    930                 935                 940

ccc gtg gtt cgt gaa ttg ctg ctg cgc gag ggc aac gat gac ccg aga   2880
Pro Val Val Arg Glu Leu Leu Leu Arg Glu Gly Asn Asp Asp Pro Arg
945                 950                 955                 960

act cag gtg gct acc cac cgt taa                                  2904
Thr Gln Val Ala Thr His Arg
                965
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<210> 6
<211> 967

<212> PRT
<213> Mycobacterium tuberculosis

<220>
<223> mmpL6 sequence and protein

<400> 6

```
Met Ser Asn His His Arg Pro Arg Pro Trp Leu Pro His Thr Ile Arg
  1               5                  10                  15

Arg Leu Ser Leu Pro Ile Leu Leu Phe Trp Val Gly Val Ala Ala Ile
            20                  25                  30

Thr Asn Ala Ala Val Pro Gln Leu Glu Val Val Gly Glu Ala His Asn
        35                  40                  45

Val Ala Gln Ser Ser Pro Asp Asp Pro Ser Leu Gln Ala Met Lys Arg
    50                  55                  60

Ile Gly Lys Val Phe His Glu Phe Asp Ser Asp Ser Ala Ala Met Ile
65                  70                  75                  80

Val Leu Glu Gly Asp Lys Pro Leu Gly Asn Asp Ala His Arg Phe Tyr
                85                  90                  95

Asp Thr Leu Leu Arg Asn Leu Ser Asn Asp Thr Lys His Val Glu His
            100                 105                 110

Val Gln Asp Phe Trp Gly Asp Pro Leu Thr Ala Ala Gly Ser Gln Ser
        115                 120                 125

Thr Asp Gly Lys Ala Ala Tyr Val Gln Val Tyr Leu Ala Gly Asn Gln
    130                 135                 140

Gly Glu Ala Leu Ser Ile Glu Ser Val Asp Ala Val Arg Asp Ile Val
145                 150                 155                 160

Ala His Thr Pro Pro Pro Ala Gly Val Lys Ala Tyr Val Thr Gly Ala
                165                 170                 175

Ala Pro Leu Met Ala Asp Gln Phe Gln Val Gly Ser Lys Gly Thr Ala
            180                 185                 190

Lys Val Thr Gly Ile Thr Leu Val Val Ile Ala Val Met Leu Leu Phe
        195                 200                 205

Val Tyr Arg Ser Val Val Thr Met Val Leu Val Leu Ile Thr Val Leu
    210                 215                 220

Ile Glu Leu Ala Ala Ala Arg Gly Ile Val Ala Phe Leu Gly Asn Ala
225                 230                 235                 240

Gly Val Ile Gly Leu Ser Thr Tyr Ser Thr Asn Leu Leu Thr Leu Leu
                245                 250                 255

Val Ile Ala Ala Gly Thr Asp Tyr Ala Ile Phe Val Leu Gly Arg Tyr
            260                 265                 270

His Glu Ala Arg Tyr Ala Ala Gln Asp Arg Glu Thr Ala Phe Tyr Thr
        275                 280                 285

Met Tyr Arg Gly Thr Ala His Val Val Leu Gly Ser Gly Leu Thr Val
    290                 295                 300

Ala Gly Ala Val Tyr Cys Leu Ser Phe Thr Arg Leu Pro Tyr Phe Gln
305                 310                 315                 320

Ser Leu Gly Ile Pro Ala Ser Ile Gly Val Met Ile Ala Leu Ala Ala
```

```
                  325                 330                 335

Ala Leu Ser Leu Ala Pro Ser Val Leu Ile Leu Gly Ser Arg Phe Gly
            340                 345                 350

Cys Phe Glu Pro Lys Arg Arg Met Arg Thr Arg Gly Trp Arg Arg Ile
        355                 360                 365

Gly Thr Ala Ile Val Arg Trp Pro Gly Pro Ile Leu Ala Val Ala Cys
    370                 375                 380

Ala Ile Ala Val Val Gly Leu Leu Ala Leu Pro Gly Tyr Lys Thr Ser
385                 390                 395                 400

Tyr Asp Ala Arg Tyr Tyr Met Pro Ala Thr Ala Pro Ala Asn Ile Gly
                405                 410                 415

Tyr Met Ala Ala Glu Arg His Phe Pro Gln Ala Arg Leu Asn Pro Glu
            420                 425                 430

Leu Leu Met Ile Glu Thr Asp His Asp Met Arg Asn Pro Ala Asp Met
        435                 440                 445

Leu Ile Leu Asp Arg Ile Ala Lys Ala Val Phe His Leu Pro Gly Ile
    450                 455                 460

Gly Leu Val Gln Ala Met Thr Arg Pro Leu Gly Thr Pro Ile Asp His
465                 470                 475                 480

Ser Ser Ile Pro Phe Gln Ile Ser Met Gln Ser Val Gly Gln Ile Gln
                485                 490                 495

Asn Leu Lys Tyr Gln Arg Asp Arg Ala Ala Asp Leu Leu Lys Gln Ala
            500                 505                 510

Glu Glu Leu Gly Lys Thr Ile Glu Ile Leu Gln Arg Gln Tyr Ala Leu
        515                 520                 525

Gln Gln Glu Leu Ala Ala Ala Thr His Glu Gln Ala Glu Ser Phe His
    530                 535                 540

Gln Thr Ile Ala Thr Val Lys Glu Leu Arg Asp Arg Ile Ala Asn Phe
545                 550                 555                 560

Asp Asp Phe Phe Arg Pro Ile Arg Ser Tyr Phe Tyr Trp Glu Lys His
                565                 570                 575

Cys Tyr Asp Ile Pro Ser Cys Trp Ala Leu Arg Ser Val Phe Asp Thr
            580                 585                 590

Ile Asp Gly Ile Asp Gln Leu Gly Glu Gln Leu Ala Ser Val Thr Val
        595                 600                 605

Thr Leu Asp Lys Leu Ala Ala Ile Gln Pro Gln Leu Val Ala Leu Leu
    610                 615                 620

Pro Asp Glu Ile Ala Ser Gln Gln Ile Asn Arg Glu Leu Ala Leu Ala
625                 630                 635                 640

Asn Tyr Ala Thr Met Ser Gly Ile Tyr Ala Gln Thr Ala Ala Leu Ile
                645                 650                 655
```

```
Glu Asn Ala Ala Ala Met Gly Gln Ala Phe Asp Ala Ala Lys Asn Asp
            660                 665                 670

Asp Ser Phe Tyr Leu Pro Pro Glu Ala Phe Asp Asn Pro Asp Phe Gln
        675                 680                 685

Arg Gly Leu Lys Leu Phe Leu Ser Ala Asp Gly Lys Ala Ala Arg Met
    690                 695                 700

Ile Ile Ser His Glu Gly Asp Pro Ala Thr Pro Glu Gly Ile Ser His
705                 710                 715                 720

Ile Asp Ala Ile Lys Gln Ala Ala His Glu Ala Val Lys Gly Thr Pro
                725                 730                 735

Met Ala Gly Ala Gly Ile Tyr Leu Ala Gly Thr Ala Ala Thr Phe Lys
            740                 745                 750

Asp Ile Gln Asp Gly Ala Thr Tyr Asp Leu Leu Ile Ala Gly Ile Ala
        755                 760                 765

Ala Leu Ser Leu Ile Leu Leu Ile Met Met Ile Ile Thr Arg Ser Leu
    770                 775                 780

Val Ala Ala Leu Val Ile Val Gly Thr Val Ala Leu Ser Leu Gly Ala
785                 790                 795                 800

Ser Phe Gly Leu Ser Val Leu Val Trp Gln His Leu Leu Gly Ile Gln
                805                 810                 815

Leu Tyr Trp Ile Val Leu Ala Leu Ala Val Ile Leu Leu Leu Ala Val
            820                 825                 830

Gly Ser Asp Tyr Asn Leu Leu Leu Ile Ser Arg Phe Lys Glu Glu Ile
        835                 840                 845

Gly Ala Gly Leu Asn Thr Gly Ile Ile Arg Ala Met Ala Gly Thr Gly
    850                 855                 860

Gly Val Val Thr Ala Ala Gly Leu Val Phe Ala Ala Thr Met Ser Ser
865                 870                 875                 880

Phe Val Phe Ser Asp Leu Arg Val Leu Gly Gln Ile Gly Thr Thr Ile
                885                 890                 895

Gly Leu Gly Leu Leu Phe Asp Thr Leu Val Val Arg Ala Phe Met Thr
            900                 905                 910

Pro Ser Ile Ala Val Leu Leu Gly Arg Trp Phe Trp Trp Pro Gln Arg
        915                 920                 925

Val Arg Pro Arg Pro Ala Ser Arg Met Leu Arg Pro Tyr Gly Pro Arg
    930                 935                 940

Pro Val Val Arg Glu Leu Leu Leu Arg Glu Gly Asn Asp Asp Pro Arg
945                 950                 955                 960

Thr Gln Val Ala Thr His Arg
                965
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<210> 7
<211> 1758
<212> DNA
<213> Mycobacterium tuberculosis

<220>
<221> CDS
<222> (1)..(1758)

<220>
<223> mmpL6 truncated sequence and protein

<400> 7

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atg agc aac cac cac cgc ccg cgg cct tgg ttg ccg cac acc atc cga    48
Met Ser Asn His His Arg Pro Arg Pro Trp Leu Pro His Thr Ile Arg
  1               5                  10                  15

cgg ctt tcg ttg ccg atc ttg ctg ttt tgg gtg ggt gtg gcc gcc ata    96
Arg Leu Ser Leu Pro Ile Leu Leu Phe Trp Val Gly Val Ala Ala Ile
             20                  25                  30

acc aat gcc gcc gtg ccg caa ttg gag gtg gtc ggg gag gcg cat aac   144
Thr Asn Ala Ala Val Pro Gln Leu Glu Val Val Gly Glu Ala His Asn
         35                  40                  45

gtc gca cag agc tcc ccg gat gac ccg tcg ctg cag gcg atg aaa cgc   192
Val Ala Gln Ser Ser Pro Asp Asp Pro Ser Leu Gln Ala Met Lys Arg
     50                  55                  60

atc ggc aag gtg ttc cac gag ttc gat tcc gac agt gcg gcc atg atc   240
Ile Gly Lys Val Phe His Glu Phe Asp Ser Asp Ser Ala Ala Met Ile
 65                  70                  75                  80

gtc ttg gaa ggc gat aag ccg ctc ggc aac gac gcc cac cgg ttc tac   288
Val Leu Glu Gly Asp Lys Pro Leu Gly Asn Asp Ala His Arg Phe Tyr
                 85                  90                  95

gac acc ctg ctc cgc aac ctt tca aac gac acc aaa cac gtc gag cac   336
Asp Thr Leu Leu Arg Asn Leu Ser Asn Asp Thr Lys His Val Glu His
            100                 105                 110

gtt cag gac ttc tgg ggc gat ccg ctg acc gcg gcc ggc tcg caa agc   384
Val Gln Asp Phe Trp Gly Asp Pro Leu Thr Ala Ala Gly Ser Gln Ser
        115                 120                 125

acc gac ggc aaa gcc gcc tac gtt cag gtc tat ctc gcc ggc aac caa   432
Thr Asp Gly Lys Ala Ala Tyr Val Gln Val Tyr Leu Ala Gly Asn Gln
    130                 135                 140

ggc gag gcg ttg tca atc gag tcc gtc gac gcg gtg cgc gac atc gtc   480
Gly Glu Ala Leu Ser Ile Glu Ser Val Asp Ala Val Arg Asp Ile Val
145                 150                 155                 160

gcc cat acg cca cca ccg gcc ggg gtc aag gcc tac gtc acc ggc gcg   528
Ala His Thr Pro Pro Pro Ala Gly Val Lys Ala Tyr Val Thr Gly Ala
                165                 170                 175

gcc ccg ctc atg gcc gat cag ttt cag gtg ggc agc aaa gga acc gcg   576
```

```
Ala Pro Leu Met Ala Asp Gln Phe Gln Val Gly Ser Lys Gly Thr Ala
            180                 185                 190

aaa gtt acc ggg ata act ctg gtt gtg atc gcg gtg atg ttg ctc ttc   624
Lys Val Thr Gly Ile Thr Leu Val Val Ile Ala Val Met Leu Leu Phe
        195                 200                 205

gta tac cgt tcc gtc gtc acc atg gtc ctg gtg ctt atc acg gtt ctt   672
Val Tyr Arg Ser Val Val Thr Met Val Leu Val Leu Ile Thr Val Leu
    210                 215                 220

att gag ttg gcc gcg gcc cgc ggg atc gtc gct ttt ctc gga aac gcc   720
Ile Glu Leu Ala Ala Ala Arg Gly Ile Val Ala Phe Leu Gly Asn Ala
225                 230                 235                 240

ggg gta atc ggg ctg tcg aca tac tcg acg aat ctg ctc aca cta ttg   768
Gly Val Ile Gly Leu Ser Thr Tyr Ser Thr Asn Leu Leu Thr Leu Leu
                245                 250                 255

gta atc gcg gcg ggc aca gac tac gcg att ttt gtc ctc ggc cgc tat   816
Val Ile Ala Ala Gly Thr Asp Tyr Ala Ile Phe Val Leu Gly Arg Tyr
            260                 265                 270

cac gag gcg cgc tac gcc gca cag gat cgg gaa acg gcc ttc tac acg   864
His Glu Ala Arg Tyr Ala Ala Gln Asp Arg Glu Thr Ala Phe Tyr Thr
        275                 280                 285

atg tat cgc ggg acc gcc cac gtc gtc ttg ggc tcg ggt ctg acc gtt   912
Met Tyr Arg Gly Thr Ala His Val Val Leu Gly Ser Gly Leu Thr Val
    290                 295                 300

gcc ggc gcg gtg tat tgc ctg agc ttt acc cgg cta ccc tat ttt caa   960
Ala Gly Ala Val Tyr Cys Leu Ser Phe Thr Arg Leu Pro Tyr Phe Gln
305                 310                 315                 320

agc ctg ggt att ccc gcc tcg ata ggg gtg atg att gcg ttg gca gcc   1008
Ser Leu Gly Ile Pro Ala Ser Ile Gly Val Met Ile Ala Leu Ala Ala
                325                 330                 335

gcg ctc agc ctg gcc cca tcc gtg ctc atc ttg ggc agt cgt ttc ggt   1056
Ala Leu Ser Leu Ala Pro Ser Val Leu Ile Leu Gly Ser Arg Phe Gly
            340                 345                 350

tgt ttc gaa ccc aag cgc agg atg agg acc agg gga tgg cgg cgc atc   1104
Cys Phe Glu Pro Lys Arg Arg Met Arg Thr Arg Gly Trp Arg Arg Ile
        355                 360                 365

ggc acg gcc atc gtg cgt tgg ccg gga ccc atc ctg gca gtg gcg tgc   1152
Gly Thr Ala Ile Val Arg Trp Pro Gly Pro Ile Leu Ala Val Ala Cys
    370                 375                 380                 --

gca att gcg gtg gtg ggt ctg ctc gcg ctg ccg gga tac aaa acg agc   1200
Ala Ile Ala Val Val Gly Leu Leu Ala Leu Pro Gly Tyr Lys Thr Ser
385                 390                 395                 400

tac gac gct cgc tat tac atg ccc gcc acc gcc ccg gcc aat att ggc   1248
Tyr Asp Ala Arg Tyr Tyr Met Pro Ala Thr Ala Pro Ala Asn Ile Gly
                405                 410                 415

tac atg gcc gcg gag cga cat ttt ccc caa gcg cgg ctg aat ccc gaa   1296
Tyr Met Ala Ala Glu Arg His Phe Pro Gln Ala Arg Leu Asn Pro Glu
```

```
            420                 425                 430

cta ctg atg atc gag acg gat cac gat atg cgc aat ccg gcc gac atg   1344
Leu Leu Met Ile Glu Thr Asp His Asp Met Arg Asn Pro Ala Asp Met
        435                 440                 445

ctc atc ttg gat agg atc gcc aag gct gtc ttc cat ctg ccc ggc ata   1392
Leu Ile Leu Asp Arg Ile Ala Lys Ala Val Phe His Leu Pro Gly Ile
    450                 455                 460

ggg ctg gtg cag gcc atg acc cgg ccg cta gga acc ccg att gac cac   1440
Gly Leu Val Gln Ala Met Thr Arg Pro Leu Gly Thr Pro Ile Asp His
465                 470                 475                 480

agc tcg ata ccg ttt cag atc agc atg caa agc gtc ggc cag att cag   1488
Ser Ser Ile Pro Phe Gln Ile Ser Met Gln Ser Val Gly Gln Ile Gln
                485                 490                 495

aat ctc aag tat cag agg gac cga gca gcc gac ttg ctg aag cag gcc   1536
Asn Leu Lys Tyr Gln Arg Asp Arg Ala Ala Asp Leu Leu Lys Gln Ala
            500                 505                 510

gaa gag ctg ggg aag acg atc gaa atc ttg cag cgc caa tat gcc cta   1584
Glu Glu Leu Gly Lys Thr Ile Glu Ile Leu Gln Arg Gln Tyr Ala Leu
        515                 520                 525

cag cag gaa ctc gcg gcc gct act cac gag caa gcc gaa agc ttt cac   1632
Gln Gln Glu Leu Ala Ala Ala Thr His Glu Gln Ala Glu Ser Phe His
    530                 535                 540

caa acg atc gcc acg gta aag gaa ctg cga gat agg atc gcc aat ttc   1680
Gln Thr Ile Ala Thr Val Lys Glu Leu Arg Asp Arg Ile Ala Asn Phe
545                 550                 555                 560

gac gat ttc ttc agg ccg att cgt agt tac ttt tac tgg gaa aag cac   1728
Asp Asp Phe Phe Arg Pro Ile Arg Ser Tyr Phe Tyr Trp Glu Lys His
                565                 570                 575

tgc tac gat atc ccg agc tgc tgg gcg ctg                          1758
Cys Tyr Asp Ile Pro Ser Cys Trp Ala Leu
            580                 585
```

<210> 8
<211> 586
<212> PRT
<213> Mycobacterium tuberculosis

<220>
<223> mmpL6 truncated sequence and protein

<400> 8

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Met Ser Asn His His Arg Pro Arg Pro Trp Leu Pro His Thr Ile Arg
  1               5                  10                  15

Arg Leu Ser Leu Pro Ile Leu Leu Phe Trp Val Gly Val Ala Ala Ile
             20                  25                  30

Thr Asn Ala Ala Val Pro Gln Leu Glu Val Val Gly Glu Ala His Asn
         35                  40                  45
```

```
Val Ala Gln Ser Ser Pro Asp Asp Pro Ser Leu Gln Ala Met Lys Arg
     50                  55                  60

Ile Gly Lys Val Phe His Glu Phe Asp Ser Asp Ser Ala Ala Met Ile
 65                  70                  75                  80

Val Leu Glu Gly Asp Lys Pro Leu Gly Asn Asp Ala His Arg Phe Tyr
                 85                  90                  95

Asp Thr Leu Leu Arg Asn Leu Ser Asn Asp Thr Lys His Val Glu His
            100                 105                 110

Val Gln Asp Phe Trp Gly Asp Pro Leu Thr Ala Ala Gly Ser Gln Ser
        115                 120                 125

Thr Asp Gly Lys Ala Ala Tyr Val Gln Val Tyr Leu Ala Gly Asn Gln
    130                 135                 140

Gly Glu Ala Leu Ser Ile Glu Ser Val Asp Ala Val Arg Asp Ile Val
145                 150                 155                 160

Ala His Thr Pro Pro Pro Ala Gly Val Lys Ala Tyr Val Thr Gly Ala
                165                 170                 175

Ala Pro Leu Met Ala Asp Gln Phe Gln Val Gly Ser Lys Gly Thr Ala
            180                 185                 190

Lys Val Thr Gly Ile Thr Leu Val Val Ile Ala Val Met Leu Leu Phe
        195                 200                 205

Val Tyr Arg Ser Val Val Thr Met Val Leu Val Leu Ile Thr Val Leu
    210                 215                 220

Ile Glu Leu Ala Ala Ala Arg Gly Ile Val Ala Phe Leu Gly Asn Ala
225                 230                 235                 240

Gly Val Ile Gly Leu Ser Thr Tyr Ser Thr Asn Leu Leu Thr Leu Leu
                245                 250                 255

Val Ile Ala Ala Gly Thr Asp Tyr Ala Ile Phe Val Leu Gly Arg Tyr
            260                 265                 270

His Glu Ala Arg Tyr Ala Ala Gln Asp Arg Glu Thr Ala Phe Tyr Thr
        275                 280                 285

Met Tyr Arg Gly Thr Ala His Val Val Leu Gly Ser Gly Leu Thr Val
    290                 295                 300

Ala Gly Ala Val Tyr Cys Leu Ser Phe Thr Arg Leu Pro Tyr Phe Gln
305                 310                 315                 320

Ser Leu Gly Ile Pro Ala Ser Ile Gly Val Met Ile Ala Leu Ala Ala
                325                 330                 335

Ala Leu Ser Leu Ala Pro Ser Val Leu Ile Leu Gly Ser Arg Phe Gly
            340                 345                 350

Cys Phe Glu Pro Lys Arg Arg Met Arg Thr Arg Gly Trp Arg Arg Ile
        355                 360                 365

Gly Thr Ala Ile Val Arg Trp Pro Gly Pro Ile Leu Ala Val Ala Cys
```

370 375 380

Ala Ile Ala Val Val Gly Leu Leu Ala Leu Pro Gly Tyr Lys Thr Ser
385 390 395 400

Tyr Asp Ala Arg Tyr Tyr Met Pro Ala Thr Ala Pro Ala Asn Ile Gly
405 410 415

Tyr Met Ala Ala Glu Arg His Phe Pro Gln Ala Arg Leu Asn Pro Glu
420 425 430

Leu Leu Met Ile Glu Thr Asp His Asp Met Arg Asn Pro Ala Asp Met
435 440 445

Leu Ile Leu Asp Arg Ile Ala Lys Ala Val Phe His Leu Pro Gly Ile
450 455 460

Gly Leu Val Gln Ala Met Thr Arg Pro Leu Gly Thr Pro Ile Asp His
465 470 475 480

Ser Ser Ile Pro Phe Gln Ile Ser Met Gln Ser Val Gly Gln Ile Gln
485 490 495

Asn Leu Lys Tyr Gln Arg Asp Arg Ala Ala Asp Leu Leu Lys Gln Ala
500 505 510

Glu Glu Leu Gly Lys Thr Ile Glu Ile Leu Gln Arg Gln Tyr Ala Leu
515 520 525

Gln Gln Glu Leu Ala Ala Ala Thr His Glu Gln Ala Glu Ser Phe His
530 535 540

Gln Thr Ile Ala Thr Val Lys Glu Leu Arg Asp Arg Ile Ala Asn Phe
545 550 555 560

Asp Asp Phe Phe Arg Pro Ile Arg Ser Tyr Phe Tyr Trp Glu Lys His
565 570 575

Cys Tyr Asp Ile Pro Ser Cys Trp Ala Leu
580 585

<210> 9
<211> 447
<212> DNA
<213> Mycobacterium tuberculosis

<220>
<221> CDS
<222> (1)..(444)

<220>
<223> mmpS6 sequence and protein

<400> 9
gtg cag ggg att tca gtg act ggc ctg gtc aaa cgc ggc tgg atg gtg 48
Val Gln Gly Ile Ser Val Thr Gly Leu Val Lys Arg Gly Trp Met Val
1 5 10 15

ctg gtt gcc gtg gcg gtg gtg gcg gtc gcg gga ttc agc gtc tat cgg 96

```
Leu Val Ala Val Ala Val Val Ala Val Ala Gly Phe Ser Val Tyr Arg
          20                  25                  30

ttg cac ggc atc ttc ggc tcg cac gac acc acc tcg acc gcc ggt ggt   144
Leu His Gly Ile Phe Gly Ser His Asp Thr Thr Ser Thr Ala Gly Gly
        35                  40                  45

gtc gcg aac gac atc aag ccg ttc aac ccc aaa cag gta acc ctc gag   192
Val Ala Asn Asp Ile Lys Pro Phe Asn Pro Lys Gln Val Thr Leu Glu
    50                  55                  60

gtc ttt ggc gct ccc gga acc gtg gca acg atc aat tat ctg gac gtg   240
Val Phe Gly Ala Pro Gly Thr Val Ala Thr Ile Asn Tyr Leu Asp Val
65                  70                  75                  80

gat gcc aca cct cgg caa gtc ctg gac acg acc ctg ccg tgg tca tac   288
Asp Ala Thr Pro Arg Gln Val Leu Asp Thr Thr Leu Pro Trp Ser Tyr
                85                  90                  95

acg atc acg acg acc ctg ccc gcg gtc ttc gcc aat gtt gtc gcg caa   336
Thr Ile Thr Thr Thr Leu Pro Ala Val Phe Ala Asn Val Val Ala Gln
            100                 105                 110

ggc gac agc aat tcc atc ggc tgc cgc atc acc gtc aac ggt gta gtc   384
Gly Asp Ser Asn Ser Ile Gly Cys Arg Ile Thr Val Asn Gly Val Val
        115                 120                 125

aag gac gaa agg atc gtc aac gaa gtg cgc gcc tat acc ttc tgc ctc   432
Lys Asp Glu Arg Ile Val Asn Glu Val Arg Ala Tyr Thr Phe Cys Leu
    130                 135                 140

gac aag tcc tca tga                                           447
Asp Lys Ser Ser
145
```

<210> 10
<211> 148
<212> PRT
<213> Mycobacterium tuberculosis

<220>
<223> mmpS6 sequence and protein

<400> 10

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Val Gln Gly Ile Ser Val Thr Gly Leu Val Lys Arg Gly Trp Met Val
  1               5                  10                  15

Leu Val Ala Val Ala Val Val Ala Val Ala Gly Phe Ser Val Tyr Arg
            20                  25                  30

Leu His Gly Ile Phe Gly Ser His Asp Thr Thr Ser Thr Ala Gly Gly
        35                  40                  45

Val Ala Asn Asp Ile Lys Pro Phe Asn Pro Lys Gln Val Thr Leu Glu
    50                  55                  60

Val Phe Gly Ala Pro Gly Thr Val Ala Thr Ile Asn Tyr Leu Asp Val
65                  70                  75                  80

Asp Ala Thr Pro Arg Gln Val Leu Asp Thr Thr Leu Pro Trp Ser Tyr
```

```
                85                  90                  95

Thr Ile Thr Thr Thr Leu Pro Ala Val Phe Ala Asn Val Val Ala Gln
            100                 105                 110

Gly Asp Ser Asn Ser Ile Gly Cys Arg Ile Thr Val Asn Gly Val Val
        115                 120                 125

Lys Asp Glu Arg Ile Val Asn Glu Val Arg Ala Tyr Thr Phe Cys Leu
    130                 135                 140

Asp Lys Ser Ser
145
  :
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<210> 11
<211> 399
<212> DNA
<213> Mycobacterium tuberculosis

<220>
<221> CDS
<222> (1)..(399)

<220>
<223> mmpS6 truncated sequence and protein

<400> 11

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ctg gtt gcc gtg gcg gtg gtg gcg gtc gcg gga ttc agc gtc tat cgg   48
Leu Val Ala Val Ala Val Val Ala Val Ala Gly Phe Ser Val Tyr Arg
  1               5                  10                  15

ttg cac ggc atc ttc ggc tcg cac gac acc acc tcg acc gcc ggt ggt   96
Leu His Gly Ile Phe Gly Ser His Asp Thr Thr Ser Thr Ala Gly Gly
            20                  25                  30

gtc gcg aac gac atc aag ccg ttc aac ccc aaa cag gta acc ctc gag   144
Val Ala Asn Asp Ile Lys Pro Phe Asn Pro Lys Gln Val Thr Leu Glu
        35                  40                  45

gtc ttt ggc gct ccc gga acc gtg gca acg atc aat tat ctg gac gtg   192
Val Phe Gly Ala Pro Gly Thr Val Ala Thr Ile Asn Tyr Leu Asp Val
    50                  55                  60

gat gcc aca cct cgg caa gtc ctg gac acg acc ctg ccg tgg tca tac   240
Asp Ala Thr Pro Arg Gln Val Leu Asp Thr Thr Leu Pro Trp Ser Tyr
65                  70                  75                  80

acg atc acg acg acc ctg ccc gcg gtc ttc gcc aat gtt gtc gcg caa   288
Thr Ile Thr Thr Thr Leu Pro Ala Val Phe Ala Asn Val Val Ala Gln
                85                  90                  95

ggc gac agc aat tcc atc ggc tgc cgc atc acc gtc aac ggt gta gtc   336
Gly Asp Ser Asn Ser Ile Gly Cys Arg Ile Thr Val Asn Gly Val Val
            100                 105                 110

aag gac gaa agg atc gtc aac gaa gtg cgc gcc tat acc ttc tgc ctc   384
Lys Asp Glu Arg Ile Val Asn Glu Val Arg Ala Tyr Thr Phe Cys Leu
        115                 120                 125
```

gac aag tcc tca tga 399
Asp Lys Ser Ser
130

<210> 12
<211> 132
<212> PRT
<213> Mycobacterium tuberculosis

<220>
<223> mmpS6 truncated sequence and protein

<400> 12
Leu Val Ala Val Ala Val Val Ala Val Ala Gly Phe Ser Val Tyr Arg
1 5 10 15

Leu His Gly Ile Phe Gly Ser His Asp Thr Thr Ser Thr Ala Gly Gly
20 25 30

Val Ala Asn Asp Ile Lys Pro Phe Asn Pro Lys Gln Val Thr Leu Glu
35 40 45

Val Phe Gly Ala Pro Gly Thr Val Ala Thr Ile Asn Tyr Leu Asp Val
50 55 60

Asp Ala Thr Pro Arg Gln Val Leu Asp Thr Thr Leu Pro Trp Ser Tyr
65 70 75 80

Thr Ile Thr Thr Thr Leu Pro Ala Val Phe Ala Asn Val Val Ala Gln
85 90 95

Gly Asp Ser Asn Ser Ile Gly Cys Arg Ile Thr Val Asn Gly Val Val
100 105 110

Lys Asp Glu Arg Ile Val Asn Glu Val Arg Ala Tyr Thr Phe Cys Leu
115 120 125

Asp Lys Ser Ser
130

<210> 13
<211> 20
<212> DNA
<213> Mycobacterium tuberculosis

<400> 13
cgttcaaccc caaacaggta — 20

<210> 14
<211> 20
<212> DNA
<213> Mycobacterium tuberculosis

<400> 14
aatcgaactc gtggaacacc 20

<210> 15
<211> 20
<212> DNA
<213> Mycobacterium tuberculosis

<400> 15
attcagcgtc tatcggttgc 20

<210> 16
<211> 20
<212> DNA
<213> Mycobacterium tuberculosis

<400> 16
agcagctcgg gatatcgtag 20

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<210> 17
<211> 20
<212> DNA
<213> Mycobacterium tuberculosis

<400> 17
ctacctcatc ttccggtcca 20

<210> 18
<211> 20
<212> DNA
<213> Mycobacterium tuberculosis

<400> 18
catagatccc ggacatggtg 20

<210> 19
<211> 2390
<212> DNA
<213> Mycobacterium tuberculosis

<220>
<221> CDS
<222> (517)..(2307)

<400> 19
gatcccgtcg ccgcggcgct ggagctggcc gccgggcccg cagccgcccc gcgcgaggtc 60

gtgctggcga gcaaagccac catgcgcgcc acagccagcc ccggatcgct ggaccttgag 120

caacacgaac tcgccaaacg cttagaactt gggccgcagg cgaaatcggt ccagtcgccc 180

gagttcgccg ctcgcttggc tgccgctcaa cacaggtagc gcctaccagc ctcgctggtt 240

tccatggcgt gccccagtcc gaagctgctg ctgcttgact ccgcgcgctg ggcccgagcg 300

cgcgctgttg tacggcccaa acggcgtgtc ggtgtacagt cgcgcgctcg cggcttcagt 360

ccggcccccc gactccggca ggcccgacgg cgcccagcgc tagcccgaag ttccccccttg 420

taggggcggg ctgagtttcg atctgtttcg tgagcaggtg tttctgtgtt caacttccct 480

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caacatgtac tcatgtatta ttgagaatag ctcggc gtg tca tcc tct gat gac   534
                                        Val Ser Ser Ser Asp Asp
                                          1               5

gct att atc gcg ctg acc gcg tgt tat aaa gta atc atg tac att acc   582
Ala Ile Ile Ala Leu Thr Ala Cys Tyr Lys Val Ile Met Tyr Ile Thr
            10                  15                  20

cgg gta ccc aac cgg gga tcc ccg ccg gcg gtg ctg ttg cgg gaa agc   630
Arg Val Pro Asn Arg Gly Ser Pro Pro Ala Val Leu Leu Arg Glu Ser
        25                  30                  35

ttc cgc gaa aac ggc aag gtc aag acg cgt acc ctg gcc aac ctc tca   678
Phe Arg Glu Asn Gly Lys Val Lys Thr Arg Thr Leu Ala Asn Leu Ser
    40                  45                  50

cgc tgg ccc gag cac aag ctg gac aga ctg gac cgg gcg ctt aag ggc   726
Arg Trp Pro Glu His Lys Leu Asp Arg Leu Asp Arg Ala Leu Lys Gly
55                  60                  65                  70

ttg ccg ccc gcg gac tgg gat cta gcc gag gcc ttc gat atc acc cgc   774
Leu Pro Pro Ala Asp Trp Asp Leu Ala Glu Ala Phe Asp Ile Thr Arg
                75                  80                  85

agc ctg ccg cac ggg cat gtg gcc gcg gtg gcc ggc acc gcc gag aag   822
Ser Leu Pro His Gly His Val Ala Ala Val Ala Gly Thr Ala Glu Lys
            90                  95                  100

ctg ggc ata ccc gag ctg atc gac ccc acc ccg tcg cgg cgg cgc aac   870
Leu Gly Ile Pro Glu Leu Ile Asp Pro Thr Pro Ser Arg Arg Arg Asn
        105                 110                 115

ctg gtg ctg gcc atg ctg atc ggg cag atc atc gag ccc gga tcg aaa   918
Leu Val Leu Ala Met Leu Ile Gly Gln Ile Ile Glu Pro Gly Ser Lys
    120                 125                 130

ctg gcg atc gcg cgc ggg ctg cgc gcc cag acc gcc acc agc acg ctg   966
Leu Ala Ile Ala Arg Gly Leu Arg Ala Gln Thr Ala Thr Ser Thr Leu
135                 140                 145                 150

ggt gcg gtg ctg ggt gtc tcg ggc gcc gat gag gac gac ctg tat gac   1014
Gly Ala Val Leu Gly Val Ser Gly Ala Asp Glu Asp Asp Leu Tyr Asp
                155                 160                 165

gcg atg gac tgg gcg ctg gag cgc aaa gac ggc atc gaa aac gcc ttg   1062
Ala Met Asp Trp Ala Leu Glu Arg Lys Asp Gly Ile Glu Asn Ala Leu
            170                 175                 180

gcc gca cgg cat ctg acc aac ggc acc ctg gtg ctc tat gac gta tcc   1110
Ala Ala Arg His Leu Thr Asn Gly Thr Leu Val Leu Tyr Asp Val Ser
        185                 190                 195

tcg gcg gcg ttc gag ggc cac acc tgc ccg ctg gga gcg atc ggg cac   1158
Ser Ala Ala Phe Glu Gly His Thr Cys Pro Leu Gly Ala Ile Gly His
    200                 205                 210

gcc cgc gac ggg gtc aaa ggc cgg ctg cag atc gtc tac ggg ctg ctg   1206
Ala Arg Asp Gly Val Lys Gly Arg Leu Gln Ile Val Tyr Gly Leu Leu
215                 220                 225                 230
```

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tgc tca ccc aag gga gcg ccg gtg gcc atc gag gtg ttc aag ggc aac   1254
Cys Ser Pro Lys Gly Ala Pro Val Ala Ile Glu Val Phe Lys Gly Asn
                235                 240                 245

acc gcc gac ccg aaa act ctg aaa gct caa atc gac aag ctc aaa acc   1302
Thr Ala Asp Pro Lys Thr Leu Lys Ala Gln Ile Asp Lys Leu Lys Thr
            250                 255                 260

cgg ttc ggg ttg acc cgc atc gcc ctg gtg ggc gat cgg ggc atg ctc   1350
Arg Phe Gly Leu Thr Arg Ile Ala Leu Val Gly Asp Arg Gly Met Leu
        265                 270                 275

act tcc gcg cgc atc cgt gac gag ctg cgt ccg gcg cac ctg gat tgg   1398
Thr Ser Ala Arg Ile Arg Asp Glu Leu Arg Pro Ala His Leu Asp Trp
    280                 285                 290

atc agc gcg ctg cgc gcc ccg cag atc aag atc ctg ctc gag gac ggg   1446
Ile Ser Ala Leu Arg Ala Pro Gln Ile Lys Ile Leu Leu Glu Asp Gly
295                 300                 305                 310

gcg ctg cag ctg tcg ctg ttc gat gag cag aac ctg ttc gag atc act   1494
Ala Leu Gln Leu Ser Leu Phe Asp Glu Gln Asn Leu Phe Glu Ile Thr
                315                 320                 325

cac ccc gac tat ccc ggt gag cgg ctg gtg tgc tgc cac aac ccc gcc   1542
His Pro Asp Tyr Pro Gly Glu Arg Leu Val Cys Cys His Asn Pro Ala
            330                 335                 340

ctg gcc gac gag cgc gcc cgc aaa cgc gcc gag ctg ctg gcg gcc acc   1590
Leu Ala Asp Glu Arg Ala Arg Lys Arg Ala Glu Leu Leu Ala Ala Thr
        345                 350                 355

gaa aag gag ctg cag gcc atc gcc gaa gcc acc cgc cgc caa cgc cgg   1638
Glu Lys Glu Leu Gln Ala Ile Ala Glu Ala Thr Arg Arg Gln Arg Arg
    360                 365                 370

ccg tta cgc ggt aca gac aag atc ggc ctg cgg gtg ggc aag gtg cgc   1686
Pro Leu Arg Gly Thr Asp Lys Ile Gly Leu Arg Val Gly Lys Val Arg
375                 380                 385                 390

aac aag ttc aag atg gcc aag cac ttt gac ctg cac atc acc gat gag   1734
Asn Lys Phe Lys Met Ala Lys His Phe Asp Leu His Ile Thr Asp Glu
                395                 400                 405

gcc ttc agc ttc acc cgc aac cag aac agt atc gcc gcc gag gcc gcc   1782
Ala Phe Ser Phe Thr Arg Asn Gln Asn Ser Ile Ala Ala Glu Ala Ala
            410                 415                 420

ctc gac ggc atc tac gtg cta cgc acc agc ctg ccc gac aac gcc ctg   1830
Leu Asp Gly Ile Tyr Val Leu Arg Thr Ser Leu Pro Asp Asn Ala Leu
        425                 430                 435

ggc cgc gac gac gtg gtg ggc cgc tac aaa gac ctc gcc gac gtc gaa   1878
Gly Arg Asp Asp Val Val Gly Arg Tyr Lys Asp Leu Ala Asp Val Glu
    440                 445                 450

cgc ttc ttc cgc acc ctc aac agc gaa ctg gac gta cgc ccc atc cgg   1926
Arg Phe Phe Arg Thr Leu Asn Ser Glu Leu Asp Val Arg Pro Ile Arg
455                 460                 465                 470

cat cgg ctg gcc gac cgg gtc cgc gcc cac atg ttc ttg cac atg ctc   1974
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His Arg Leu Ala Asp Arg Val Arg Ala His Met Phe Leu His Met Leu
                475                 480                 485

tcc tac tac atc agc tgg cac atg aaa caa gcc ctg gcc cca atc ctg   2022
Ser Tyr Tyr Ile Ser Trp His Met Lys Gln Ala Leu Ala Pro Ile Leu
            490                 495                 500

ttc acc gac aac gac aaa ccc gcc gcc gcc gcc aaa cgc gcc gac ccc   2070
Phe Thr Asp Asn Asp Lys Pro Ala Ala Ala Ala Lys Arg Ala Asp Pro
        505                 510                 515

gtc gcg cca gcc caa cgc tcc gac gaa gcg ctg aac aag gca gca cgc   2118
Val Ala Pro Ala Gln Arg Ser Asp Glu Ala Leu Asn Lys Ala Ala Arg
    520                 525                 530

aaa cgc acc gaa gac aac caa ccg gtg cac agc ttc acc agc ctg ctc   2166
Lys Arg Thr Glu Asp Asn Gln Pro Val His Ser Phe Thr Ser Leu Leu
535                 540                 545                 550

acc gac ctg gcc acc atc tgc gcc aac tac atc caa ccc aca gac gac   2214
Thr Asp Leu Ala Thr Ile Cys Ala Asn Tyr Ile Gln Pro Thr Asp Asp
                555                 560                 565

ctg cca gca ttc acc aaa acc acc acc ccc acc ccc aca caa cgg cgc   2262
Leu Pro Ala Phe Thr Lys Thr Thr Thr Pro Thr Pro Thr Gln Arg Arg
            570                 575                 580

gcc ttc gac cta ctg gcc gtt tcc cac cgc cac ggc ctg gcg tag       2307
Ala Phe Asp Leu Leu Ala Val Ser His Arg His Gly Leu Ala
        585                 590                 595

tcagtaccga accacaaatg cccaggtcaa cgacacaaac cgcgccggat caggggaac 2367

ttcgggctag ccgggcgcgc cgg                                        2390


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Val Ile Met Tyr Ile Thr Arg Val Pro Asn Arg Gly Ser Pro Pro Ala
             20                  25                  30

Val Leu Leu Arg Glu Ser Phe Arg Glu Asn Gly Lys Val Lys Thr Arg
         35                  40                  45

Thr Leu Ala Asn Leu Ser Arg Trp Pro Glu His Lys Leu Asp Arg Leu
     50                  55                  60

Asp Arg Ala Leu Lys Gly Leu Pro Pro Ala Asp Trp Asp Leu Ala Glu
 65                  70                  75                  80

Ala Phe Asp Ile Thr Arg Ser Leu Pro His Gly His Val Ala Ala Val
                 85                  90                  95

Ala Gly Thr Ala Glu Lys Leu Gly Ile Pro Glu Leu Ile Asp Pro Thr
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                100                 105                 110

Pro Ser Arg Arg Arg Asn Leu Val Leu Ala Met Leu Ile Gly Gln Ile
        115                 120                 125

Ile Glu Pro Gly Ser Lys Leu Ala Ile Ala Arg Gly Leu Arg Ala Gln
    130                 135                 140

Thr Ala Thr Ser Thr Leu Gly Ala Val Leu Gly Val Ser Gly Ala Asp
145                 150                 155                 160

Glu Asp Asp Leu Tyr Asp Ala Met Asp Trp Ala Leu Glu Arg Lys Asp
                165                 170                 175

Gly Ile Glu Asn Ala Leu Ala Ala Arg His Leu Thr Asn Gly Thr Leu
            180                 185                 190

Val Leu Tyr Asp Val Ser Ser Ala Ala Phe Glu Gly His Thr Cys Pro
        195                 200                 205

Leu Gly Ala Ile Gly His Ala Arg Asp Gly Val Lys Gly Arg Leu Gln
    210                 215                 220

Ile Val Tyr Gly Leu Leu Cys Ser Pro Lys Gly Ala Pro Val Ala Ile
225                 230                 235                 240

Glu Val Phe Lys Gly Asn Thr Ala Asp Pro Lys Thr Leu Lys Ala Gln
                245                 250                 255

Ile Asp Lys Leu Lys Thr Arg Phe Gly Leu Thr Arg Ile Ala Leu Val
            260                 265                 270

Gly Asp Arg Gly Met Leu Thr Ser Ala Arg Ile Arg Asp Glu Leu Arg
        275                 280                 285

Pro Ala His Leu Asp Trp Ile Ser Ala Leu Arg Ala Pro Gln Ile Lys
    290                 295                 300

Ile Leu Leu Glu Asp Gly Ala Leu Gln Leu Ser Leu Phe Asp Glu Gln
305                 310                 315                 320

Asn Leu Phe Glu Ile Thr His Pro Asp Tyr Pro Gly Glu Arg Leu Val
                325                 330                 335

Cys Cys His Asn Pro Ala Leu Ala Asp Glu Arg Ala Arg Lys Arg Ala
            340                 345                 350

Glu Leu Leu Ala Ala Thr Glu Lys Glu Leu Gln Ala Ile Ala Glu Ala
        355                 360                 365

Thr Arg Arg Gln Arg Arg Pro Leu Arg Gly Thr Asp Lys Ile Gly Leu
    370                 375                 380

Arg Val Gly Lys Val Arg Asn Lys Phe Lys Met Ala Lys His Phe Asp
385                 390                 395                 400

Leu His Ile Thr Asp Glu Ala Phe Ser Phe Thr Arg Asn Gln Asn Ser
                405                 410                 415

Ile Ala Ala Glu Ala Ala Leu Asp Gly Ile Tyr Val Leu Arg Thr Ser
            420                 425                 430
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Leu Pro Asp Asn Ala Leu Gly Arg Asp Asp Val Val Gly Arg Tyr Lys
435 440 445

Asp Leu Ala Asp Val Glu Arg Phe Phe Arg Thr Leu Asn Ser Glu Leu
450 455 460

Asp Val Arg Pro Ile Arg His Arg Leu Ala Asp Arg Val Arg Ala His
465 470 475 480

Met Phe Leu His Met Leu Ser Tyr Tyr Ile Ser Trp His Met Lys Gln
485 490 495

Ala Leu Ala Pro Ile Leu Phe Thr Asp Asn Asp Lys Pro Ala Ala Ala
500 505 510

Ala Lys Arg Ala Asp Pro Val Ala Pro Ala Gln Arg Ser Asp Glu Ala
515 520 525

Leu Asn Lys Ala Ala Arg Lys Arg Thr Glu Asp Asn Gln Pro Val His
530 535 540

Ser Phe Thr Ser Leu Leu Thr Asp Leu Ala Thr Ile Cys Ala Asn Tyr
545 550 555 560

Ile Gln Pro Thr Asp Asp Leu Pro Ala Phe Thr Lys Thr Thr Thr Pro
565 570 575

Thr Pro Thr Gln Arg Arg Ala Phe Asp Leu Leu Ala Val Ser His Arg
580 585 590

His Gly Leu Ala
595